THE UNIVERSITY OF LIVERPOOL

ANNALS
OF
TROPICAL MEDICINE AND PARASITOLOGY

ISSUED BY THE

LIVERPOOL SCHOOL OF TROPICAL MEDICINE

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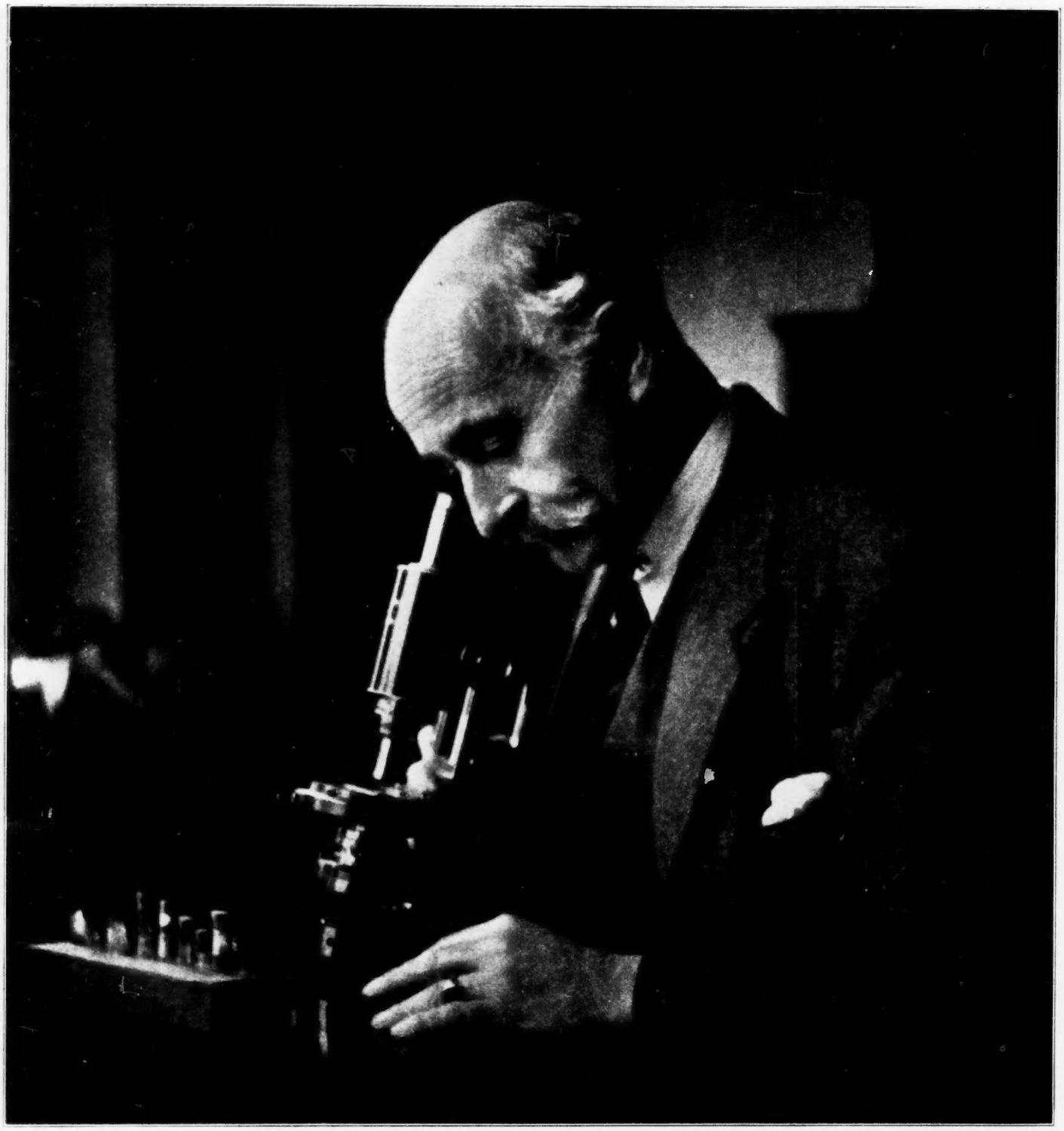

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The following courses of instruction are given by the Liverpool School of Tropical Medicine each year :—

(1) Two courses for the Diploma in Tropical Medicine, commencing on the 5th January and the 1st October, 1931. The D.T.M. examinations are held in December and March.

(2) Two courses for the Diploma in Tropical Hygiene, commencing on the 12th January, and the 22nd April, 1931. The D.T.H. examinations are held in March and July.

(3) Two courses in Veterinary Parasitology, commencing on the 5th January and the 1st October, 1931.

DIPLOMA IN TROPICAL MEDICINE

This Diploma shall be awarded only to candidates who possess a qualification to practise Medicine recognised for this purpose by the University, and who present satisfactory certificates of having attended approved courses of study, and pass the prescribed examination.

DIPLOMA IN TROPICAL HYGIENE

This Diploma can only be taken by those who have already obtained the D.T.M. of the University of Liverpool.

' The course for this Diploma will not be conducted unless at least five applications are received, and no application for admission can be considered later than December 21st and March 31st respectively.'

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MICRO-ORGANISMS IN THE GUT OF *GLOSSINA PALPALIS*

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INTRODUCTION

Carpenter (1912), in his report on the bionomics of *Glossina palpalis*, described an apparent incompatibility between trypanosomes and 'certain long bacilli' present in unstained preparations of the gut of these flies. Prates (1928) also noted these long bacilli, and stated that his observations gave him a similar impression of the association between them and trypanosomes.

In 1928 I was led by these observations to investigate the long bacilli more fully, and soon reached the conclusion that the bacteria noticed by Carpenter and by Prates were undoubtedly the 'bacteroids,' 'symbionts' or 'symbiotes'* described by Stuhlmann (1907) and again, in connection with the digestion of blood, by Roubaud (1919). I found that the distribution of the bacteroids was more restricted than I had at first realised and that they could be easily missed in teasing up the gut in the course of an ordinary dissection.

* The symbiotic nature of these structures not having been proved, throughout this paper I have used the word bacteroid as being non-committal.

Cultures were made from wild and laboratory-bred flies under various conditions, and on different media, but the only growths obtained were a few isolated ones presumably accidental contaminations from the air or from the exterior of the flies. The bacteroids did not grow and no other organisms were seen in the stained smears from the gut.

Since these preliminary experiments were made, several contradictory statements have been published concerning the bacterial flora of the tsetse-fly's gut, and the incompatibility with trypanosomes, described by Carpenter, if not actually accepted, has certainly not been explained away.

Thus, as regards micro-organisms in the gut, Wigglesworth writes (1929, p. 292), ' Various other micro-organisms (bacilli, staphylococci, encapsulated diplococci) were present in small numbers '; and again (1930, p. 562), ' The tsetse-fly feeding upon a sterile diet, has in its gut a very restricted flora.'

Lloyd (1930, p. 540), says : ' The bacteria in the gut vary greatly in number, and several species occur,' and again, ' Flies which have been kept under laboratory conditions for some time are in general more heavily infested with gut bacteria than wild flies.' He also implies that flies can become infected with bacteria from dirty fly-boxes by testing the walls of the box with their proboscis, but does not give the evidence for these statements.

Hindle, in the discussion that followed the reading of Wigglesworth's paper (1930, p. 574), suggested that bacteriolysins present in the tsetse-fly might be responsible for ' *the usual bacterial sterility of the gut* ' (my italics).

Waterson, in the same discussion, said : ' There was no doubt that in the laboratory one could make insects take up bacteria or similar organisms although they did not seem to do this under natural conditions.'

Regarding the incompatibility between long bacilli and trypanosomes, Lloyd (1930), referring to Carpenter's work on *G. palpalis*, says : ' Carpenter (1912) noted in his dissection of wild flies that those with a large number of bacteria rarely contained trypanosomes,' and appears to accept this incompatibility as one of the factors influencing the rate of infection of the gut with trypanosomes.

Lastly, Wigglesworth (1930, p. 562), discussing Prates' statement

mentioned above, says : ' The description given of these organisms, however, agrees with that of the intra-cellular " bacteroids " which we have seen occur in every fly.' But he does not explain why these ' long bacilli ' were observed in only a limited percentage of the flies examined.

In an endeavour to explain some of these contradictions, a further series of cultural experiments was made, using larger numbers of flies. These experiments, which form the larger part of this paper, tend to show that the *living* tsetse-fly, even if kept in grossly contaminated boxes has few, if any, micro-organisms in its gut culturable on ordinary media.

OBSERVATIONS AND EXPERIMENTS

I. The Localisation of the Bacteroids.

In investigating the bacteroids and their relation to trypanosomes, it was first necessary to determine their position in the gut. Wigglesworth (1929) has described the minute anatomy of the gut of *Glossina*, including the exact position of the bacteroid zone, but in May, 1928, when my experiments were begun, the only description available was in Roubaud's study of the nutrition of tsetse-flies.

Roubaud (1919, p. 512), described the distribution of the cells containing the bacteroids as follows : ' L'auteur allemand Stuhlmann, dans son bon travail consacré particulièrement a *Gl. fusca* (*brevipalpis*) a fait connaître l'existence, *dans toute la longeur* de ce qu'il nomme l'intestin moyen (Mitteldarm), d'une zone particuliére suivant laquelle l'epithélium se trouve localement et irrégulièrement épaissi, présentant macroscopiquement *des taches ou des bandes* de couleur plus blanche que le reste de l'organe ' (my italics).

I made stained films from different regions of the gut and found that the bacteroids were limited to a small portion, 3-4 mm. long, at the posterior end of that part which is relatively straight and narrow and is usually free from obvious blood. As noted by Wigglesworth (1929), the zone of giant cells containing bacteroids is far more localised than indicated by Roubaud. Under favourable conditions it can be seen with the naked eye as an opaque patch which, with a hand lens, appears as an elongated white ring. Lester and Lloyd (1928) figure this ring (diagram I, page 40) as ' mesenteric

gland.' They describe it thus : ' A double band of cells of a special nature in the anterior part of the mesenteron form a gland of unknown function.' The publication containing this diagram reached this laboratory shortly after the approximate position of the bacteroids had been found and it proved useful in defining their more exact localisation.

The above brief account of the localisation of the bacteroids is included in this paper for completeness and as a summary of my own observations. I do not intend in any way to detract from the very full and lucid description given by Wigglesworth, but rather to give an independent confirmation of its accuracy.

II. The Presence of ' Long Bacilli ' in Wild and Laboratory-bred Flies.

Having seen that the zone in which the bacteroids occur is very limited in extent, it is easy to explain the low* percentage of flies in which Carpenter, and Prates observed ' long bacilli.' Unless the existence of the bacteroids and their localisation is known, the giant-cell area can easily be missed in teasing up the gut and in that case the ' long bacilli ' will not be liberated. Even if this is realised, the bacteroids are not always demonstrable in every fly in a series, for the amount of blood in the gut and the stage of digestion reached sometimes makes it difficult to find the bacteroid zone, and moreover when dead flies are examined, if some time has elapsed since death, the dryness of the fly may lead to the loss during dissection of this particular portion of the gut.

Thus, in a series of laboratory-bred flies, stained smears of the gut were examined, and bacteroids were seen in 37 out of 42, or only 88 per cent. In each of these flies the gut was dissected and its entirety verified as far as possible. It was then teased up thoroughly and smeared on a slide, fixed in 5 per cent. formalin and stained with methylene blue.

In another series, wild flies were used and preparations of the gut were examined unstained as well as stained. In the unstained preparations, bacteroids were seen in 50 per cent., in the stained films in 91 per cent. (It appeared to be somewhat easier to demon-

* See Dr. Carpenter's letter to the Editor of the *Transactions of Tropical Medicine and Hygiene*, **24**, No. 2, 255. This does not, however, affect the explanations here given.

strate bacteroids in wild flies probably because they are not fed as regularly as laboratory-bred flies, and so have less blood in the gut.)

In 6·6 per cent. of these wild flies, *T. grayi* was found ; *in every fly in which this trypanosome was seen bacteroids were also present.*

In all these films and in some two hundred others examined from time to time in smaller batches, *the bacteroids were the only organisms seen*, except in films made from flies some time after death. If precautions are not taken to avoid contaminating the preparation with organisms from the exterior of the fly, numerous other bacteria may be included. This occurs more frequently in flies dying in the boxes than in those killed immediately before dissection. It is here assumed that the ' long bacilli ' and the bacteroids are identical. The fact that no other organisms were seen in the gut supports this assumption, and moreover, if smears from the entire gut are compared with those made from the giant-cell zone, it will be seen that the organisms in the two sets of films do not differ in morphology.

The bacteroids are non-motile, without capsules and curved in a characteristic way that is easy to recognise but difficult to describe. They stain well with simple stains, are gram-negative and not acid-fast. With methylene blue or with Giemsa's stain they stain unevenly and show clear areas that are so distinct that a long bacillus may look like a chain of shorter ones. The average size is 7·4 × ·5 microns, but some individuals are much longer (48 microns) and resemble filaments of Actinomyces (Streptothrix) except that they are not branched. Roubaud (1919, p. 516) considers them to be yeasts because of the formation of buds (' bourgeons '). He also regards them as related to the symbiotic yeasts observed in various insects by other observers, e.g., Karawaiew (1899) and Escherich (1900).

Nothing resembling buds has been seen in any of the preparations examined, though some of the organisms have somewhat club-shaped ends. Glaser (1930), working with the cockroach *Periplaneta americana*, has isolated the ' symbiotes ' or ' rickettsia ' found in the bacteriocytes (mycetocytes) among the fat tissue. He gives figures of these bacteria-like forms and, mainly on their morphology, regards them as belonging to the genus *Corynebacterium*. The bacteroids of *Glossina* resemble the micro-organisms depicted by Glaser, and there seems to be no more evidence for placing them

among the yeasts than for the suggestion that they, too, are *Corynebacteria*.

III. Attempts at Cultivation of the Bacteroids.

There are several difficulties to be overcome in making cultures from the gut of *Glossina*. The dissection of the gut entire cannot be done rapidly but entails considerable manipulation and exposure, particularly in recently-fed flies. During this manipulation there is the risk of contamination from the air and from the exterior of the fly. If the gut is removed from the abdomen by pressure with a needle, organisms from the outer surface are bound to be included.

The following technique was adopted after trying several other methods of sterilisation such as rapid flaming, dipping in alcohol and then flaming, or alcohol alone.

The wings and legs of all the flies to be cultured are first removed with scissors. A slide is flamed and left face downwards with one end raised so that the sterile surface is protected from dust and from contact with the bench. A fly is then held by the proboscis in sterile forceps and dipped in and out of tincture of iodine twenty times. It is then rinsed in a 10 per cent. solution of sodium thiosulphate in water till the iodine colouration is removed. After being thoroughly washed in a stream of sterile saline from a wash bottle, the fly is laid on the sterile surface of the prepared slide. The tip of the abdomen is then snipped off with a pair of sterile scissors and the abdominal contents pressed out with a flamed needle. As much as possible of the adherent fat body is removed by rapid dissection with a pair of sterile needles and the gut is then picked up with one of them and dropped into the culture medium.

If it is desired to culture the bacteroid zone only, the gut has to be dissected more thoroughly and its entire length made clear. It is then washed by shaking it in a tube of sterile saline, placed upon another sterile slide and the bacteroid zone divided off with a needle and put into the culture medium. Forty flies in all were cultured in this way, both aerobic and anaerobic methods being used, and the cultures kept at room temperature on the bench or in an incubator at 37° C. (The mean maximum temperature at Entebbe during 1928 was 79° F. (26·1° C.), the mean minimum was 63·2° F. (17·3° C.).

The following media were used :—

(1) Nutrient agar and broth.
(2) Glucose broth.
(3) N.N.N. medium.
(4) Levindthal's agar.
(5) Glucose broth enriched with extract of tsetse gut.

The nutrient agar was made of chopped ox heart with peptone and adjusted to a reaction of pH. 7·4.

Levindthal's agar was the medium used by Bruce White (1921), in his investigation of the bacterial flora of the bee. It contained 5 per cent. sheep's blood.

The gut extract was made by grinding up the mid-gut in saline with clean sand and then filtering through a Seitz asbestos filter. The extract made in this way was shown to be sterile and was used in two different dilutions, the first with approximately one gut per tube of broth, the second three times this strength. Cultures were also made in the indiluted extract.

The bacteroids did not grow in any of these media. In five cultures growths were obtained that were considered to be contaminations. They were entirely different in morphology from the bacteroids and similar to organisms grown from the outer surface of the flies and from the walls of the fly-boxes.

They included spore-bearing bacilli of the subtilis and mesentericus group, staphylococci and most commonly several kinds of unidentified gram-negative bacteria.

Several hanging-drop culture-preparations were made from the bacteroid zone. Escherich (1900), using 1 per cent. glucose in water to cultivate the symbiotic yeasts from *Anobium paniceum*, kept them alive for several weeks and noted definite multiplication. Accordingly, 1 per cent. glucose solution was tried for the bacteroids of *Glossina* but no sign of increase was observed. The organisms remained unchanged for two weeks in a moist chamber at room temperature. The gut extract, described above, was used in a similar preparation with the same result.

IV. Experimental Infection of Flies with Bacteria :—

(*a*) by feeding them on infected blood ;

(*b*) attempts at infection by contaminating the fly-boxes with a known organism.

(*a*) These experiments were commenced with the idea of comparing the bacteroids, *in situ* in the gut, with bacteria of known morphology. Later, however, it was realised that the recovery by culture of a known micro-organism, previously introduced into the gut, would show that the antiseptic used to sterilise the outside of the fly did not penetrate to the interior of the gut in quantity sufficient to prevent growth.

Experiment 1. 28.vii.28.

Flies fed on an infected rabbit.

A rabbit was injected intravenously with 2 c.c. of a saline suspension from an agar slope of *B. paratyphosus* B (equivalent to half the growth on the slope in 24 hours).

A box of 50 ' clean '* laboratory-bred flies was applied to it as soon as possible after the injection. Cultures were made daily in nutrient broth, from sample flies killed with chloroform and from any flies dying overnight. The exterior of the flies was sterilised by iodine, as described in Section III. Sub-cultures were made on agar from all broth tubes showing growth and were then tested by agglutination with a specific serum. Stained films were made from the gut contents of some of the flies and the distinction between the paratyphoid bacilli and the bacteroids was clear. *B. paratyphosus* B was recovered from flies up to the 10th day after the infecting feed, although three feeds on ' clean ' animals were given in the meantime. A negative culture, from the last surviving fly, was obtained on the 11th day.

Experiment 2. 15.viii.28.

A similar experiment to 1 but using *B. typhosus* instead of *B. paratyphosus* B.

The organism remained in the flies only as long as the 5th day after the infecting feed with one ' clean ' feed on the 4th day. Negative cultures were obtained on the 6th, 7th, 8th, 9th, and 10th days.

Experiment 3. 23.x.28.

Using blood *in vitro* instead of a living animal.

Sterile blood from a guinea-pig was defibrinated and put into a sterilised glass funnel, the mouth of which was covered with a piece of guinea-pig's skin, freshly removed from the animal and used with the hairy side outwards. 5 c.c. of a saline suspension of an agar growth of *B. paratyphosus* B was then added and the funnel placed on the gauze top of a fly-box containing ' clean ' laboratory-bred flies. The flies fed readily and well. (This method is a modification of that used by Rodhain (1913), and is described by Prates (1928, p. 175).)

* Throughout this paper the expression ' clean ' flies is used for flies that have never fed ; on the other hand, a ' clean ' animal means one that has not been infected.

In this experiment, paratyphoid bacilli were recovered from sample flies not later than the 4th day after the infecting feed. Two negative cultures were obtained on the 8th and 9th days.

EXPERIMENT 4. 25.i.29.

A similar experiment to 3 but using *B. prodigiosus*.

A box of flies was fed on defibrinated sheep's blood infected with *B. prodigiosus** (5 c.c. of a broth culture to 25 c.c. of blood). The same procedure was used except that a length of glass tubing 2 inches in diameter and about 4 inches long was used instead of the glass funnel. The tube was plugged with wool at one end and a piece of guinea-pig's skin was tied over the other.

Cultures of *B. prodigiosus* were obtained up to the 24th day after the infecting feed. Flies dying on the 34th and 39th days were negative and so was the last fly of the series which was killed on the 40th day. The flies were given seven ' clean ' feeds on a cock between the infecting feed and the termination of the experiment.

B. prodigiosus and bacteroids were both seen in stained films from the appropriate portion of the gut, the bacteroids being apparently unaffected by the presence of the extraneous organisms.

All flies dying during the course of the experiment and subsequently found to be infected with *B. prodigiosus* were extremely fragile. Very gentle traction on the wings or legs led to their separation from the body of the fly. The broth culture from one of these flies was plated out on agar but no organism other than *B. prodigiosus* was found. (The fragility described above has been noticed several times in flies dying during the course of routine transmission experiments at Entebbe. It will be referred to again at the end of Section V, under the heading of Controls.)

EXPERIMENT 5. 9.ii.29.

Another experiment using *B. paratyphosus* B and *T. rhodesiense*.

A box of 50 flies was fed, through skin, on a tube of defibrinated blood infected with *B. paratyphosus* B. After two ' clean ' feeds, the flies were fed once, on 18.ii.29, on a monkey infected with *T. rhodesiense*, and thereafter on a ' clean ' animal. Before this date, *B. paratyphosus* B was recovered from flies dying on the 5th, 7th, and 9th day, after the first infecting feed. After the feed on the monkey infected with trypanosomes, fresh preparations, as well as cultures, were made from 18 of the flies. Trypanosomes were seen in two of these, but bacterial cultures from the same two flies were negative. *B. paratyphosus* B, however, was recovered from flies up to and including the 30th day after the first infecting feed, negative cultures being obtained on the 32nd, 37th, 40th, and 43rd days.

EXPERIMENT 6. 21.iii.29.

A similar experiment to 5, but using *B. prodigiosus* instead of *B. paratyphosus* B.

The flies in two boxes, 50 in each, were fed through rat's skin on sheep's blood infected with *B. prodigiosus*. All flies that did not appear to have fed were killed and the remaining 62 were given one ' clean ' feed after the infecting feed. Between 23.ii.29 and 28.ii.29, they were fed three times on a monkey infected with *T. rhodesiense* and thereafter on a ' clean ' animal.

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* This organism was used because it was easily recognisable in culture. It was isolated from a brown iridescent scum on the surface of water in the Botanic Gardens, Entebbe. It is a short motile gram-negative bacillus, which forms a bright pink ring at the margin of the surface of a broth culture, in 48 hours at room temperature. This colour subsequently extends throughout the medium. The broth culture is toxic to guinea-pigs, 0·5 c.c. of a 48 hour culture killing a 500 grm. animal in less than 24 hours.

As the flies died they were sterilised, dissected, and the gut expressed on to a slide. Each gut was divided into two parts, one being examined under a coverslip for trypanosomes and motile bacilli, and the other cultured. None of the flies contained trypanosomes, and *B. prodigiosus* was recovered up to the 26th day after the initial infecting feed. The last surviving fly, killed and cultured on the 27th day, was free from *B. prodigiosus* infection. 'Fragility' was noted in all the dead flies in this experiment, in some being so marked that dissection was difficult.

(*b*) attempts at infections by contaminating the fly-boxes with a known organism.

In the preceding section evidence was given to show that a bacterial infection in the gut of *G. palpalis* may persist as long as thirty days. It may be argued that this infection did not persist from the time of the initial infecting feed but occurred in a fly which did not feed at that time but infected itself later, through its proboscis, by palpating the wall of the box contaminated with the faeces of other infected flies.

The following experiments suggest that this does not occur.

Experiment 7. 24.i.29.

Attempt to infect flies with *Torula rosea.*

Ten 'clean' recently hatched and unfed flies were kept in a box with one of its walls covered by a sheet of blotting paper kept wet with a broth culture of *T. rosea.*

Three of these flies were cultured without sterilisation and the test organism was recovered from their exterior. Two of the three were dropped whole into broth, the head and proboscis of the third were removed and cultured.

Four more of the ten flies were sterilised and dissected, and cultures were made from the gut. Three of these were killed before sterilisation, the other one had died. No growth was obtained from any of the four.

Of the remaining three flies, which were too dry to dissect, two were washed in saline. The deposit after centrifugalisation of this saline contained cells of *Torula.*

A culture from the blotting paper on 28.i.29, at the conclusion of the experiment, was positive, showing that the *Torula* was still alive.

Experiment 8. 7.v.30.

Since it is possible that *T. rosea* cannot grow in the gut even if it is taken up by the fly, the experiment was repeated using *B. prodigiosus*, which was shown in experiments 4 and 6 to be capable of growth in the gut.

Forty-eight 'clean' flies, hatched on 5.v.30, were starved on 6.v.30. On 7.v.30 a sheet of blotting paper was placed inside the box and wetted with a 24-hour broth culture of *B. prodigiosus.*

On 8.v.30 the flies were starved and the paper re-wetted with culture. On 9.v.30 the paper was re-wetted and five dead flies were labelled DNS (dead not sterilised) 1, DNS 2, DS (dead sterilised) 1, DS 2, and DSW (dead sterilised, cultured whole).

Fly DNS 1 was dropped whole without sterilisation, into broth. *Result:* Pink growth.

Fly DNS 2 was teased up without sterilisation and cultured. *Result:* Pink growth. (The saline left on the slide after this fly was cultured contained motile bacilli.)

Fly DS 1. The exterior was sterilised and the gut cultured. *Result :* Growth of moulds only.

Fly DS 2. Treated in the same way as D 1. *Result :* Pink growth.

Fly DSW. The exterior was sterilised and the fly cultured whole. *Result :* No pink growth but growth of *B. subtilis*.

On 10.v.30 five flies died, two of them, DS 3 and DS 4, were sterilised and cultures made from the gut. *Result :* Pink growth in both.

The paper was re-wetted for the last time and the flies were fed on a ' clean ' cock. Three more flies died after feeding. They were not cultured ; coverslip preparations made from the gut showed bacteroids but no motile organisms.

On 11.v.30 four flies died and were thrown away, since four ' dead ' flies had already been cultured.

On 12.v.30 two more flies died and were thrown away. The 29 flies left were killed with chloroform. One of them, NS (not sterilised), was cultured without sterilisation, and a control culture was made from the blotting paper. *Result :* Pink growth in both cultures.

The other 28 flies were sterilised, dissected, and the gut cultured. *Result after one week at room temperature :* No growth in 24 of the cultures, single colonies of moulds in three, and growth of *B. prodigiosus* in one only. (The fly from which this positive culture was obtained, may have been a dead one missed when the others were removed, or it may have been moribund and thus liable to infection. With this exception the only positive cultures were obtained from dead flies or from killed flies that had not been sterilised. *All the other cultures made from ' killed ' flies were negative.*)

V. Cultural Experiments with Wild and Laboratory-bred Flies.

It was thought desirable to make cultures from a larger number of flies than was used in the preliminary experiments in 1928, to see if *G. palpalis* was naturally infected with any micro-organisms. Accordingly the following series of cultures was made.

Experiment 9. 26.v.30.

A series of cultures from laboratory-bred flies kept under routine conditions.

Fifty flies, hatched out on 2.v.30, were first fed on 4.v.30 on a ' clean ' fowl. They were kept in similar boxes alongside the flies used in the routine transmission experiments at the Laboratory, under the same conditions of temperature and moisture, and were fed in the ordinary way without cleaning or sterilising the skin of the animal or bird on which they fed. They were kept in the boxes for 24 days, so that they had as much chance of becoming infected from the soiled boxes as the routine experimental flies. During this time 8 flies died and were rejected, and the remainder, that had last fed on 24.v.30, were divided into two groups for convenience in making cultures.

In both groups the flies were killed with chloroform, sterilised as usual and the gut dissected out. Each gut was divided into two parts, one of which was cultured aerobically and the other anaerobically.

The aerobic culture tubes contained ordinary nutrient broth, the anaerobic tubes glucose broth with a piece of asbestos at the bottom to form a nidus. The

anaerobic tubes were previously heated to boiling, cooled and inoculated, and then melted vaseline was poured on the surface as a seal.

Group 1 contained 23 flies, aerobic and anaerobic cultures being made from all of them.

Group 2 contained 19 flies which were kept till the next day, 27.v.30. One fly that had died overnight was sterilised, dissected, and cultured as above, to see if there was any difference between ' dead ' flies and ' killed ' flies (cf. experiment 8). The rest of the flies were killed and two of them, as a control, were cultured, whole, without sterilisation. The first of these, NS 1 (not sterilised), was cultured aerobically, the other, NS 2, anaerobically. The remaining 16 flies were sterilised, dissected, and cultured in the same way as the first group.

Result after 9 days' growth at room temperature. There was no growth in the anaerobic culture from the dead fly, the aerobic culture gave a scanty growth of a staphylococcus. Of the other 39 aerobic tubes, 35 were quite clear and free from growth, two contained moulds only, one a white staphylococcus, and one *B. subtilis*. All the anaerobic cultures were free from growth, except, of course, NS 2. NS 1 and 2 gave growths of an organism similar to the one predominant on the plates from the interior of the boxes. (See Controls below.)

Thus, out of 39 aerobic cultures made from 39 ' killed ' flies, 35 (89·7 per cent.) were free from growth.

EXPERIMENT 10. 24.vi.30.

A series of cultures from wild flies caught on the Lake shore.

Wild flies caught on 23.vi.30, were placed in two boxes. The flies in the first box, 24 in number, were chloroformed, sterilised, dissected, and cultured on 24.vi.30, using the same technique as in Experiment 2. The second box, containing 25 flies, was kept unfed till next day, when five flies were found dead and were thrown away. The remaining 20 were killed, sterilised, dissected, and cultured in the same way as before.

Result after 10 days' incubation at room temperature. 35 of the aerobic tubes and all 44 anaerobic tubes were free from growth. Of the remaining nine aerobic cultures, one contained moulds only, two gram-positive spore-bearing bacillus, and six a staphylococcus (two *S. aureus* alone, two *S. albus* alone, one *S. aureus* mixed with a gram-positive spore-bearing bacillus and one *S. albus* mixed with a gram-negative bacillus).

Thus, 35 out of 44 aerobic cultures from wild flies (79·5 per cent.) were free from growth. (The probable reason for the larger number of wild flies giving growth from the gut, as compared with laboratory-bred flies, is that the gut in most of the wild flies was nearly empty and therefore more difficult to free from the fat body and other organs, so that more time was taken in the dissection and, consequently, there was greater exposure to contamination. In both experiments the anaerobic cultures were inoculated first, which may explain why there were no contaminations among them.)

CONTROLS. 27.v.30.

As a control, some of the commoner organisms found in the boxes and on the surface of the flies, were isolated. The medium used was ordinary agar, that is to say, the same medium as was used in Experiments 9 and 10. The interior of a fly-box was washed with broth which was afterwards plated out on nutrient agar, both aerobic and anaerobic cultures being made.

(1) The predominant organism on these plates was a short bacillus, almost a coccus, which was non-motile and gram-negative. Its growth on agar was confluent and had a strong fishy smell.

Less numerous on the plates were :—

(2) A Staphylococcus with cream-coloured raised colonies.

(3) A minute bacillus, non-motile and gram-negative, giving orange-yellow colonies on agar. These colonies tended to dry up very rapidly and the organism did not live more than a week on agar when first isolated.

(4) A slender bacillus, also gram-negative, giving sulphur-yellow colonies.

All these organisms, except the Staphylococcus, grew anaerobically as well as aerobically.

Other organisms which have been grown, from time to time, from the boxes or from the exterior of the fly, are described below. No attempt at a complete identification of any of these bacteria was made, a rough comparison between those found on different occasions being thought sufficient.

(5) A spore-bearing bacillus of the type *B. subtilis*, giving a wrinkled scum on liquid media.

(6) A Staphylococcus with bright orange colonies.

(7) A gram-positive coccus with large (7 mm. in 48 hours at room temperature) colonies, very sticky and paint-like.

(8) A filamentous organism, *Actinomyces* sp. (Streptothrix) with hard raised colonies with a white bloom on them.

(9) A rapidly-motile short diplo-bacillus, gram-negative and giving a yellow-green fluorescence in broth culture.

This last organism sometimes occurs in dead flies, which it renders extremely fragile. Such flies are difficult to dissect cleanly for they fall to pieces with the lightest touch. The bacilli are seen in enormous numbers throughout the preparation, and are in rapid motion. *They have, however, never been found in flies killed for dissection.* (One of the sources from which I isolated this organism, was a preparation of Dr. Duke's, made from a ' fragile ' fly, to which he called my attention. He tells me that he has noted a similar motile bacillus in flies, usually containing partially digested blood, that have died in the course of his transmission experiments. It also, was associated with fragility in the flies. He further states that a passage in his paper, ' On the effect on the longevity of *G. palpalis* of trypanosome infections,' Duke (1928, p. 26), in which he says, ' Heavy bacterial infections of the intestine are seen from time to time and I have noted that these only very rarely occur in flies infected with trypanosomes, but there is no evidence that these bacteria are pathogenic to the fly,' does not, however, refer to this organism, but to the bacteroids of whose true nature he was at the time ignorant.)

DISCUSSION

1. Why is the gut of *G. palpalis* apparently sterile ? There are two possibilities (*a*) no organisms are taken in, and (*b*) they are destroyed as they are entering or after they have entered the gut.

(*a*) The normal food of the tsetse-fly is sterile blood and there is no unequivocal evidence that, in nature, it sucks up water or any other fluid likely to be contaminated with micro-organisms. The experiments described in this paper tend to show that *G. palpalis* does not pick up infection from its surroundings, though possibly other species

of *Glossina* may do so. Why the fly does not infect itself through its proboscis when palpating objects or when feeding on an animal whose skin and hair are heavily contaminated with micro-organisms is far from clear. It is obvious that there must be micro-organisms on the tip of the fly's proboscis after it has touched an infected surface, but it is not so certain that they are necessarily sucked up into the gut. It is possible that there is a downward flow of saliva which washes them away.

To throw light on the nature of the movements of fluid in the proboscis of *Glossina*, a continuation of Experiment 1 was made. The flies which had fed, on 28.vii.28, on a rabbit immediately after it had been injected intravenously with a culture of *B. paratyphosus* B were starved for one day and then fed on a 'clean' rabbit. Positive cultures were obtained from sample flies killed on 28.vii.28 and 29.vii.28, showing that the infection had been transferred to the flies, which were again fed on the same 'clean' rabbit on 2.viii.28 and 6.viii.28, with three days of starvation in between. Blood cultures from this rabbit, on 30.vii.28, ten minutes after the flies had fed on it, and again on 6.viii.28, were negative.

Similarly, the flies in Experiment 2 fed on blood infected with *B. typhosus* did not transmit the infection to a 'clean' rabbit.

In these experiments it was possible that some few bacilli were inoculated into the rabbit by the flies but failed to develop owing to the bactericidal power of the blood in the living animal, so a third experiment was performed in which this effect was reduced, to some extent, by using defibrinated blood *in vitro*. The flies in Experiment 3 which, on 23.x.28, had been fed on blood infected with *B. paratyphosus* B, were starved for one day and then fed on *sterile* citrated blood in a second tube. (Two sample flies killed on 24.x.28 were shown to contain *B. paratyphosus* B in the gut.) Cultures made from the second tube of blood on 25.x.28, as a control before the flies were fed on it, on 26.x.28, and on 29.x.28, were all negative. It would thus appear that there is no regurgitation between the gut and the proboscis, but these experiments alone are insufficient to warrant a final conclusion on this point. Until more information is available, all that can be said is that transference of the infection did not take place.

(*b*) It is possible that the saliva of the tsetse fly is bactericidal

and so has an effect in preventing the entry of living micro-organisms, over and above the purely mechanical process suggested above. I can find no reference to the presence in the saliva of any such substance as a bacteriolysin though Lloyd (1928) has demonstrated an anti-coagulin in it and Wigglesworth (1929) has shown that it contains no digestive ferments.

If the lines of defence suggested above are ineffective and organisms do enter the gut, they may still be destroyed there by bacteriolysins. There seems to be no direct evidence for the presence of bacteriolysins in the gut, though there is a possibility suggested by the occurrence of ' symbiotic ' bacteria in the gastric caeca of certain insects, for example in *Dysdercus* sp., the cotton-stainer. According to Glasgow (1914), these bacteria are invariably present in *Dysdercus* and, furthermore, are hereditarily transmitted, being present in the gut of the embryo. Their function is believed to be the inhibition of foreign bacteria.

In *G. palpalis* the part played by the bacteroids in the digestion of blood, though considered important by Roubaud, has been somewhat discredited by Wigglesworth. It is conceivable that they may secrete bacteriolysins and so keep the gut sterile, as the ' symbiotes ' of *Dysdercus* are believed to do. It seems to me, however, more likely that the function of the bacteroids of *Glossina* is similar to that of other symbiotes, namely that suggested by Reichenow as quoted by Ries (1930, p. 294), ' dass alle Insekten wie all Wirbeltiere niedere pflanzliche Mikroorganismen im Haushalt nötig haben, und dass deshalb Formen mit steriler Nahrung—dahin würden die Läuse als ausschliessliche Blutsauger gehören—der Gefahr des Bakterien-mangels durch eine erbliche Symbiose vorbeugen.'

There is another piece of evidence, also indirect, for the presence of bacteriolysins in the gut of *Glossina*, in the work of Duncan (1926), who has shown that there is a thermostabile bactericidal substance in the gut of *Stomoxys calcitrans*, and in some other insects and arachnids. It is possible that there is a similar substance in the gut of *Glossina* which may account for its usual sterility. According to Duncan, the commoner pathogenic bacteria when taken up by an arthropod under natural conditions, do not survive in it for long. In support of this statement, he adduces the work of Graham-Smith (1913), who fed flies on cultures of *B. prodigiosus*, *B. typhosus* and other organisms.

(The species of fly used is not given by Duncan and the original paper was not available.) *B. prodigiosus* survived in the crop for about seventeen days and *B. typhosus* was recovered from the gut contents up to the sixth day. These figures may be compared with the results of my experiments which are summarised in the following table :—

TABLE I. EXPERIMENTS WITH FLIES FED ON BLOOD INFECTED WITH BACTERIA.

Experiment	Organism	Day on which last positive culture obtained	Days when cultures were negative
1	*B. paratyphosus* B ...	10th	11th only.
2	*B. typhosus*	5th	6th, 7th, 8th, 9th, and 10th.
3	*B. paratyphosus* B ...	4th	8th and 9th.
4	*B. prodigiosus*	24th	34th, 39th, and 40th.
5	*B. paratyphosus* B ...	30th	32nd, 37th, 40th, and 43rd.
6	*B. prodigiosus*	26th	27th only.

Throughout this paper the words ' infected ' and ' infection ' have been used, somewhat illogically perhaps, in referring to flies that had fed on infected blood. The duration of this ' infection ' cannot be directly determined for one cannot tell, without killing it, whether or not an individual fly has the organism in its gut. With this proviso, the conclusion seems justified that in Experiments 4 and 5, as in Graham-Smith's experiments, the infection has died out in the flies.

2. Have the bacteroids or bacteria in the gut any action on trypanosomes ?

The statement that the bacteroids of *Glossina* have an inhibiting effect on trypanosomes in the gut has already been mentioned. It is clear that the bacteroids, being present in every fly, cannot be absolutely incompatible with trypanosomes but it is logically possible though admittedly unlikely, that they may have a constant adverse action on trypanosomes and may thus be one of the factors which determine the low percentage of flies that become infected when fed

on an infected animal even if there are numerous trypanosomes in its peripheral blood.

As regards other bacteria, it is well known that if they are present in the culture medium they prevent the growth of trypanosomes. Reasons have been given above for thinking there are usually no bacteria in the gut of *Glossina*, so the question of their effect on any trypanosomes in it does not arise. If, however, bacteria are experimentally introduced into the gut of flies subsequently fed on an animal infected with trypanosomes, they appear to have some inhibiting effect. Experiments 5 and 6 give an indication of this.

Rodhain (1913) fed flies on blood and other fluids through animal membranes, in order to measure the amount of blood taken up by a single fly and also to discover the infecting forms of the trypanosomes carried by *G. morsitans*. In the description of his experiments he states that it is essential to keep both the blood and the apparatus sterile if it is desired to feed several flies in succession, for if they take up blood or other fluid already contaminated with a certain number of bacteria, the flies will soon be killed by the multiplication of the bacteria in the gut. In this connection, it is interesting to note that blood, infected with *B. prodigiosus*, was readily taken up by flies when it was fresh, but on the next day was refused by them and even by hungry flies.

Rodhain's statement raises the question of the effect of a bacterial infection of the gut on the mortality of *G. palpalis*. That the presence of bacteria in the gut is not necessarily fatal is shown by Experiments 4, 5 and 6, in this paper, where infected flies lived at least twenty-four days. It may be that *G. morsitans*, the species with which Rodhain worked, is more readily killed by intestinal infections than *G. palpalis*, or the organisms, with which the blood in his tubes was contaminated, may have been more virulent. The flies in my experiments were not *soon* killed by the organisms in the infected blood, though the mortality over the period of the experiments seemed somewhat increased, especially in Experiment 6, in which *B. prodigiosus* was the infecting organism. There are so many factors influencing the mortality of flies in captivity that in order to obtain significant figures, larger numbers of flies must be used and the experiments so planned as to allow comparison with existing estimates of the mortality of untreated flies.

The insistence of Rodhain on the necessity for sterility in the apparatus should be compared with the conclusion arrived at after consideration of Experiments 7 to 10. If these are accepted it will be seen that there is no need for great cleanliness in the cages in which the flies are kept during the routine transmission experiments.

In conclusion, my thanks are due to Dr. Duke, the Director of the Institute, for much helpful criticism and advice.

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SUMMARY

1. Experiments are described which show why certain observers, who recorded the presence of ' long bacilli ' in the gut of approximately 20 per cent. of wild *G. palpalis*, did not find these bacilli in *every* fly.

2. Reasons are given for considering these ' long bacilli ' to be identical with the intra-cellular bacteroids present in every fly, and therefore the incompatibility which these workers believed to exist between the ' long bacilli ' and trypanosomes, is shown to be apparent only and due to the methods used in demonstrating the bacilli.

3. Attempts were made to cultivate the bacteroids on various media though without success.

4. ' Clean ' laboratory-bred flies were fed on blood infected with known bacteria and the bacteria afterwards recovered in cultures from the gut of the flies. The duration of the ' infection ' is shown to vary with different organisms and with some a point was reached when no further positive cultures were obtained. The longest time an ' infection ' remained in a fly without killing it was thirty days.

5. Evidence is given to show that tsetse-flies do not pick up bacterial infection even from grossly contaminated surroundings. The extreme cleanliness of the cages sometimes advocated in transmission experiments is therefore not essential.

6. Cultures from numbers of flies show that during life the gut is apparently free from micro-organisms other than the bacteroids.

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THE ELIMINATION OF *AEDES ARGENTEUS* POIRET AS A FACTOR IN DENGUE CONTROL IN QUEENSLAND

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The transmission of Dengue Fever and the widespread misery which this disease produces is, fortunately, for Australia confined to the north-east, stretching as far south as the northern portions of New South Wales : limits noted by Ferguson (1927). The elimination of the mosquito which carries this disease is a health measure of primary importance. Its control is only possible provided an educated and sympathetic public co-operate. The objective of intensive control of inhabited areas is to insist on proper screening of rain-water tanks against *Aedes argenteus* Poiret (*Stegomyia fasciata* F.—*Aedes aegypti* (L.) Dyar), and the elimination of all kinds of water-holding rubbish. Unfortunately, *Aedes argenteus* has a tendency to depart from its usual habits and by choosing places of compulsion, sometimes of an unusual and grotesque nature, considerably increases the possibility of larvae being overlooked, because they are not so easily detected. In areas under control it becomes more and more noticeable that most species of mosquitos, when chased from one place to another, show a tendency to do this ; hence, the removal of hundreds of water-holding rubbish that an intensive campaign brings about, tends to force *Aedes argenteus* to forsake its natural habitats, and to frequent such unusual breeding-places as flower-pots in ' bush ' houses and places of a similar nature. It must also be noticed that this mosquito selects horse troughs, gutters, only if cemented, and even gully-traps, if the water is not polluted ; occasionally *A. argenteus* will be found in water on the ground, but this is the exception rather than the rule. Its love for

water in which paint brushes are left soaking almost suggests a depraved taste. *A. argenteus* is making steady inroads into all the inland towns of Queensland, and most assiduously follows the railway lines. Its presence in any community is evidence of the neglect of Local Authorities in carrying out the provisions of the Health Acts. It is a most persistent species, and hibernates mainly in the egg stage. In spite of the fact that the temperature in parts of Australia falls below 60° F. in the winter months, numbers of adults pass the winter in warm secluded spots. In addition, all the developmental stages of this species are capable of surviving the unfavourable season of winter in Queensland, provided that the water-holding rubbish in which they breed is found in sheltered places. The control of the species would be very materially assisted if only the Queensland winters were severe enough to kill the insects right off.

AEDES ARGENTEUS AND WATER TEMPERATURES

In conducting intensive control in Brisbane it has constantly been forced upon our attention that though low temperatures occur during the winter months, the larvae survive, mostly lying prone on the bottom of the jar during the colder portions of the day, and becoming active again with the increase of temperature throughout the day.

July 28th, 1929, was a typical winter day, and the temperatures recorded on that date give a fair indication of the fluctuations which can take place.

JAR CONTAINING ABOUT HALF GALLON OF WATER (LEFT ON THE VERANDAH).

Time	Temperature of the water	Observations of Larval behaviour
6 a.m.	43° F.	Motionless on bottom of jar.
7 a.m.	43° F.	Motionless on bottom of jar.
8 a.m.	53° F.	Few movements; lying on bottom.
9 a.m.	73° F.	Very active.
10 a.m.–12 noon	74° F.	Very active—vessel in sun.
1 p.m.	76° F.	Very active—vessel in sun.
2 p.m.	70° F.	Very active—vessel in sun.
3 p.m.	67° F.	Still on surface.
4–6 p.m.	66° F.	Still on surface.
7 p.m.	62° F.	Still on surface.
8 p.m.	60° F.	On the bottom.
9 p.m.	57° F.	Lying on bottom.

From these figures, taken from a number of records, it would appear that a low temperature of the type here recorded serves only to incapacitate the larvae for the time being, but that as soon as the temperature rises no ill effects seem to be noticeable, such activity diminishing again with the advance of night. With the decrease of temperature the larvae seem forced to leave the surface, at all events for the time being; though if maintained for any length of time death must necessarily result—what the thermal death point may be is yet to be determined!

Variations of Temperatures between top and bottom of tank.

Readings taken during one week in August show a consistent difference, which in winter time would have a bearing upon mosquito activity.

	Bottom		Top	
	6 a.m.	6 p.m.	6 a.m.	6 p.m.
Minimum	60° F.	66° F.	62° F.	64° F.
Maximum	66° F.	74° F.	70° F.	74° F.

VIABILITY OF EGGS

In the viability of their eggs, *Aedes argenteus* and *Aedes* (*Finlaya*) *notoscriptus* Skuse have much in common. It is a curious thing that the former species should have acquired so many characteristics for safeguarding it from destruction. That the eggs of these two species should be capable of prolonged desiccation is demanded by the types of breeding-places that they select, breeding-places that not only dry up quickly, but are subject to prolonged periods of drought. The factors involved in regulating the hatching of such eggs are numerous, as Buxton and Hopkins (1927) and Roubaud (1929) point out. Under favourable conditions the eggs usually hatch within a period of three days. The viability of the eggs of this species is of considerable importance in anti-mosquito control. Eggs are frequently laid in roof gutters immediately after periods of rain and if, owing to dryness, they remain attached to the sides of the gutter for any length of time, the first storm may carry them away into the tank where contact with the water and other stimuli cause the eggs to hatch within a day or so. This frequently explains the

presence of larvae in tanks, etc., at times when there seems to be no reasonable ground for expecting them to be so plentiful. The larvae of *A. argenteus* and *A. notoscriptus* can be superficially distinguished by their individualistic wriggle which, at first is difficult to differentiate, and by the power of *Aedes notoscriptus* to remain under water for a longer period of time, without coming to the surface for air.

NATURAL BREEDING PLACES

This species was originally a tree breeder. With the advent of civilisation it has learnt to select vessels and as it is a species evidently now dependent upon human blood it confines its breeding-places entirely to those in the vicinity of houses. It is interesting to find, however, rare instances where this mosquito will breed in water upon the ground. The occasions are particularly rare and can be explained in such a manner as to convince the most sceptical that such breeding-places are only accidental, instances of which are given by Hamlyn-Harris (1928). In dealing with mosquitos it is important to distinguish the breeding-places which are chosen selectively, and those chosen only because none other are available. In dealing with the domestic species such idiosyncrasies are more noticeable than with sylvan types whose natural habitats remain undisturbed for longer periods of time.

OVIPOSITION DEPENDENT UPON PROXIMITY TO BLOOD SUPPLY

It is interesting to note that this mosquito does not occur at any distance from domestic surroundings, as can be shown by its avoidance of rot-holes in trees situated at some distance from its preferential breeding-places. This fact alone makes the elimination of this species a purely domestic affair, and it is always difficult to make the householder understand that the control of swamps and waterholes can exercise no influence whatever upon the prevalence of Dengue Fever. It is an extraordinary thing how slowly this idea sinks into human consciousness. Dunn (1927) has given us some observations on the oviposition of *Aedes aegypti* (L.) in relation to distance from habitations in Nigeria.

When a survey of trees situated in the public thoroughfares and parks of Brisbane is made, it is extremely noticeable what a small range of flight this mosquito seems to possess and it may be laid down

as an almost unfailing rule that *Aedes argenteus* will only select preferential vessels containing water *in the immediate vicinity of its blood supply*. *Aedes argenteus* breeds less and less in tree cavities. Its place is usually taken, when the breeding-places are situated at a greater distance from houses, by *Aedes notoscriptus* Skuse, essentially a tree breeder but a mosquito showing a distinct tendency to become domestic. It is not frequent for these to associate together and when they do it is more often on the border line where their two respective breeding-places tend to overlap, but for the rest we find a distinct demarcation in the localities selected by them. A further proof of this, if it were required, lies in the fact that *Aedes argenteus* when found in cemeteries usually occurs in close proximity to dwellings only, and that as the graves are found further and further away from the latter *Aedes notoscriptus* selects them quite naturally. *Culex fatigans*, on the other hand, shows none of these delicate distinctions, hence its association with other mosquitos on a much larger scale. When these facts are taken into consideration it will be more easily understood why the water in vessels on the graves though differing but little in the main should breed mosquitos accustomed naturally to different conditions (Hamlyn-Harris, 1929).

HOUSE TO HOUSE PATROL

The value of house to house inspections is demonstrated in a most forceful way by the accompanying details. No other methods of dealing with the elimination of breeding-places of *A. argenteus* have been found to be so effective. Three instances of an instructive nature are given.

INTENSIVE SURVEY :

Original inspections	Time of year	Needing control	Details of larvae found		
			A. argenteus		*C. fatigans*
			(*a*) In tanks	(*b*) In other water-holding vessels	
Block 56—572 houses visited	March, 1927	296	127	78	98
542 houses re-visited	May, 1927 After a lapse of 3 months	97	30	15	21

This block, situated in the heart of the south side of the city, is composed of 25 per cent. business premises, 20 per cent. residential buildings, boarding houses, flats, etc., and 55 per cent. middle-class homes. The breeding-places consisted mainly of :—tins, troughs, bedroom jugs, blacksmiths' tubs, ant-saucers, roof-gutters, jars, gulley-traps, tyres, cement mixers, drains, liquid manure, old baths, boilers, paint-pots, flower-pots, and saucers.

During the course of three months, the percentage of premises needing control, viz., 51·7 per cent. was reduced to 17·8 per cent., whilst the number of defective tanks, 22·2 per cent., was reduced to 5·5 per cent.

Original inspections	Time of year	Needing control	Details of larvae found		
			A. argenteus		*C. fatigans*
			(*a*) In tanks	(*b*) In other water-holding vessels	
Block 57—520 houses visited	October, 1927	367	76	38	94
597 houses re-visited	January, 1928 After a lapse of 4 months	36	10	9	14

The foregoing block is composed of 20 per cent. business premises, 10 per cent. boarding-houses, etc., and 70 per cent. private houses, mostly small, situated close to the heart of the city. Breeding-places were mainly as follows :—blacksmiths' tubs, tins, liquid manure, tubs, jugs, tyres, troughs, oil-drums, gutters, boilers, gulley-traps, tanks, saucers, baths, old motor bodies.

In this case the percentage of places needing control, viz., 70·5 per cent., was reduced to 6 per cent., whilst the re-inspection of tanks showed a reduction from 14·6 per cent. to 1·7 per cent.

Original inspections	Time of year	Needing control	Details of larvae found		
			A. argenteus		*C. fatigans*
			(*a*) In tanks	(*b*) In other water-holding vessels	
Block 52—522 houses visited	October, 1927	246	61	23	49
460 houses re-visited	January, 1928 After a lapse of 4 months	18	3	3	6

This is a residential area containing only a few shops, but consisting generally of large houses in the main streets and cottages in the side streets, 60 per cent. were of the cottage type. With a mixed class of residents there was little opposition to the Campaign, the majority carrying out recommendations without any written

notice. Possibly 60 per cent. were owners of their own occupied premises, and in the poorer localities, 50 per cent. possessed no tanks at all.

The following were the principal breeding-places :—kerosene tins, pots, etc., paint-tins, horse troughs, gulley-traps, tanks, barrels, milk churns, plant-pots, water-jugs, tree cavities, pineapple plants.

During the summer months when mosquitos are always at their worst, the number of premises needing control was 44·5 per cent. This figure was reduced to 3·9 per cent. between the first and second inspections, whilst the percentage of tanks, 11 per cent., pronounced ineffective, was reduced to 0·65 per cent. This speaks volumes for the value of intensive control.

INCIDENTAL TO INTENSIVE WORK

There is an interesting phase to the question of intensive control which I may be pardoned for mentioning here.

Senior-White, writing from Calcutta, draws attention to the enormous difference in the number of mosquitos collected from flats occupied by married couples, as compared with those of the bachelors. The ladies' flats were, of course, full of cushions, curtains, and other hangings, whilst the bachelors' flats were more or less bare, and harboured comparatively no mosquitos.

ECONOMIC LOSS IN WAGES AND EFFICIENCY

The following figures give in a conclusive manner some idea of the economic loss accruing to the employees of nine firms trading in Brisbane during the period of the Dengue Epidemic in 1926.

DIRECTLY ATTRIBUTABLE TO DENGUE FEVER DURING JANUARY, FEBRUARY, MARCH, AND APRIL (part), 1926.

Firms	No.	Loss in wages	Percentage of employees affected
		£ s. d.	
Not mentioned by name, but by numbers	1	438 4 8	65·6
	2	569 2 7	23·8
	3	213 17 4	17·0
	4	200 0 0	20·0
	5	220 0 0	20·0
	6	303 0 0	26·0
	7	500 0 0	35·0
	8	130 0 0	24·0
	9	1451 0 0	15·3

The total monetary loss to the employees of nine firms alone, taken at random, was £4,025 4s. 7d., involving an average of 27·4 per cent. employees. Even allowing for slight errors in compilation these figures cannot be ignored.

From the consideration of these figures, complete as far as they go, it will be seen how far reaching such sickness becomes even when the epidemic is comparatively mild and mainly confined to coastal districts. These figures, of course, make no attempt to estimate the actual loss of efficiency due to other causes, inertness, loss of vigour, and depression for long periods after so-called convalescence and to general inevitable disorganisation, which is general on such occasions. The number of cases of suicide directly attributable to Dengue after such epidemics is overwhelming.

WHERE DOES OUR DENGUE COME FROM?

The presence of Dengue in Queensland is therefore a serious matter of economic importance to all parts of the State, and it is of some moment to discover, if possible, the source of the infection in Australia. That it is not endemic is evident from the fact that we only get it in epidemiological form every so many years, usually 3, 7 or 10, and *no* authentic cases of Dengue occur during the intervals between the outbreaks. It is possible that Australia may get its Dengue Fever from India and Ceylon, via the East. There is some evidence to show this. Brisbane suffered from its last epidemic in 1926 and during 1925-1926, there were outbreaks in both Hong Kong and the Federated Malay States. During these years India's principal seaports suffered, increasing in intensity from 1921-1925. No other epidemics of Dengue appear to have occurred elsewhere.

Previous epidemics show similar connections : when, for instance, in 1905 a severe epidemic broke out in Brisbane, probably 90 per cent. of the population becoming infected, the only traceable source of infection came from Shangai which had been badly affected shortly before in January and February, 1905. The Isthmian Canal Zone also suffered badly that year, as well as in 1904. The last epidemic in the Philippines was in 1906 ; in Fiji in 1913, and in Java in 1906, which precludes these places as possible sources of infection from being considered at all in this connection. With regard to the Philippines (Siler, Hall, and Hitchens, 1926) it would appear that

cases of Dengue among the natives are not always reported; not so among the white foreigners. This may be due to the fact that the disease may occur in an atypical form which is not so easily recognised. In Swatow, China, on the other hand, the outbreaks are very similar to those which occur in Queensland. As may be seen in the following extract from a letter of Dr. C. J. Brangwin, to the writer, dated 25th February, 1929: 'We always get timely warning of the approach, as the papers tell us of the start of an epidemic in India.'

If there is any foundation for such contentions, a serious responsibility rests upon India to take up the work of the elimination of *Aedes argenteus*, more especially in the coastal towns. If Australia is to be kept free from Dengue it is evident that at present it will have to rely on its own efforts to prevent it.

P. J. Barraud (1928) in dealing with the distribution of Dengue Fever in India, makes no reference to any control measures adopted: it may, therefore, be assumed with justification that in India, in towns such as Calcutta, no serious effort whatever is made to control mosquitos.

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NOTE: Barraud gives a very comprehensive list of references with regard to Dengue in India and the East, and Siler, Hall and Hitchens on the subject generally.

LEPTOSPIRA ICTERO-HAEMORRHAGIAE IN RATS IN TRINIDAD, B.W.I.

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The island of Trinidad, situated 10° north of the equator and about 7 miles off the coast of South America, has been visited in the past by outbreaks of fever diagnosed clinically as yellow fever. Sporadic cases as well as localized outbreaks of jaundice of unknown etiology are common. Cases of illness in human beings with fever, jaundice, haemorrhage, muscular pains and the other clinical features of infection with *Leptospira ictero-haemorrhagiae,* are encountered especially during the rainy season in various parts of the island. The discovery of *Leptospira ictero-haemorrhagiae* in local rats is therefore of some interest and significance.

Brazil is the locality nearest to Trinidad where infected rats have been found according to the list given by Ruge (1928). There, of seventy-seven animals examined, 19·5 per cent. were infected. In Trinidad, during the period 1929-30, one hundred and twenty rats were examined for *Leptospira ictero-haemorrhagiae.* The animals were caught alive in the city of Port-of-Spain, killed by chloroform, the kidneys removed, emulsified in normal saline and examined by dark ground illumination and 1 c.c. of the emulsion injected intra-peritoneally into guinea-pigs weighing about 250 grammes. The results are tabulated on page 32.

Dark ground examination. Twenty-six (26·6 per cent.) of one hundred and twenty rats in Trinidad examined by dark ground illumination were found infected. In four of these positive cases the animals had been kept for six months in a cage in the laboratory, where infection was almost impossible, thus indicating that in Trinidad infection may persist for at least six months.

Guinea-pig inoculation. The pathological results produced by inoculation of guinea-pigs with pathogenic *Leptospira-haemorrhagiae* are well known. In Trinidad, in thirty instances (27·0 per cent.),

the guinea-pig developed the typical symptoms and post mortem appearances of the disease, e.g., fever, jaundice and haemorrhage in subcutaneous tissue, congestion of supra-renal, and haemorrhagic mottling of the lungs—' the butterfly lungs ' of the Japanese, etc. In some cases jaundice was much more marked than haemorrhage. Death invariably took place on sixth or seventh day after inoculation. In two instances where dark field examination revealed leptospirae, the inoculated guinea-pig failed to develop the disease due either to the low virulence of the organism or to the high resistance of the guinea-pig.

Total number of rats examined = 120.

	Positive	Negative	Percentage infected.
By dark ground illumination	32 Sex M. 18 F 14	88 Sex M. 34 F. 54	26·6
By guinea-pig inoculation (9 rats not inoculated)	30 Sex. M. 16 F. 14	81 Sex. M. 30 F. 51	27·0

Weight groups of 90 rats as suggested by Middleton.

Group in grammes	0–75	75–150	150–225	225–300	300–375
Number examined	6	35	25	17	7
Percentage infected	17	20	32	23	56

Sex. Males showed a higher incidence of infection than females—a result which agrees with that of Middleton, who says, ' presumably the male, being the more active, would stand a better chance of becoming infected.'

Species. One hundred and five of these rats were *R. norvegicus* and fifteen *R. rattus*. Of the latter, only two males showed leptospira.

Weight. The older and heavier animals show the heaviest incidence of infection, due most likely to more frequent exposure to infection.

Artificial cultivation. Both Fletcher's and Ungermann's media readily yielded cultures when sown with blood or liver juice taken twenty-four to forty-eight hours before the death of an inoculated guinea-pig. After death, contamination by invading organisms was common.

Growth was obvious from the sixth day and was most abundant and the organisms showed the greatest activity when the medium was covered with sterile paraffin.

SUMMARY

1. *Leptospira ictero-haemorrhagiae* was found in thirty-two out of one hundred and twenty (26·6 per cent.) rats examined in Trinidad.

2. Of these strains, all but two were pathogenic to guinea-pigs and produced the usual symptoms followed by death in six to seven days.

3. The incidence of infection was highest in the older and heavier rats.

4. Cultivation on artificial media was readily obtained.

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THE PAIL CLOSET AS AN EFFICIENT MEANS OF CONTROLLING HUMAN HELMINTH INFECTION AS OBSERVED IN TURA PRISON, EGYPT, WITH A DISCUSSION ON THE SOURCE OF ASCARIS INFECTION

BY

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I. INTRODUCTION

The relationship of soil pollution to the spread of Ancylostoma, Ascaris and Trichocephalus infection is an established fact. Schistosomiasis infection on the other hand depends upon the pollution of fresh water courses with human excreta. It is evident that any method of limiting or preventing soil and water pollution will result in partial or complete control of these infections as well as other bacterial, protozoal and helminthic infections.

Any method of conservancy will certainly be better than promiscuous pollution. The types of latrines and methods of disposal of human sewage are numerous, but all aim at prevention of infection with bacterial diseases and occasionally the final utilization of the sewage for fertilizing agricultural lands. The standard of purification accepted everywhere is essentially a bacterial standard as well as a measure of the degree of disintegration of the organic matter. The final fate of helminth ova did not receive any attention till lately. The author (Khalil, 1921), examined the efficiency of different methods of sewage disposal followed in British Guiana from the helminthological standpoint and arrived at the conclusion that the biological method of sewage disposal by the Septic Tank does not eliminate the danger of ancylostoma and ascaris infection. These results were confirmed by McVail (1922). Haslam (1929), questioned the value of these results, stating that the number of days and detail of working of the septic tanks in question were not stated. This however does not invalidate the results of these tanks which, by the known standards of sanitation, were in perfect working order. They were erected in a Government Hospital and their effluent contained viable ova. Giglioli in discussing Haslam's paper mentioned that he examined the effluent of tanks at MacKenzie and did not find ova. For obvious reasons negative results in such an investigation have little or no value in the presence of positive results. The results depend upon the presence of infection amongst those using the tank, the intensity of that infection and how often the tank is used, etc.

The final fate of the helminth ova is only of importance as regards the final disposal of the sewage or effluent. It is obvious that if such a fluid containing viable ova is used for irrigation in fields or let into open streams accessible to human beings grave results may ensue. This is actually what is being done in some localities.

The success of any method of sewage disposal in controlling helminth infection depends primarily upon the efficiency of enforcing it and the degree of elimination of soil and water pollution. The case of Tura Prison is unique in that, on the one hand it demonstrates the efficiency of the simple pail system when rigorously enforced and the danger on the other hand of utilizing the raw sewage for agricultural purposes.

It is obvious that amongst the general population in tropical countries sanitation cannot be enforced as in a prison. On the other hand, it is important to observe that the low initial cost and proper maintenance in clean condition of any type of latrine are primary and all important factors amongst the poor population ensuring its adoption and use.

It is claimed by many authorities that the inhabitants in certain localities are averse to the use of any form of latrine and prefer the open ground. This is not exact, as often the latrines are not well maintained so that in a short time they become muddy, dirty and smelling, so that any reasonable individual will not use them. They often, as has been repeatedly demonstrated, become a source of Ancylostoma infection.

II. TURA PRISON AND THE MEANS OF SEWAGE DISPOSAL IN IT

Tura Convict Prison lies about 13 kilometres south of Cairo on the right bank of the Nile. At this locality the eastern range of hills approach so close to the river bank that there is practically no cultivation.

The prisoners are employed in cutting Lime stones from the hills, transporting the stones to the river bank, where they are put in ships or in railway trucks. The number of prisoners varies from 2,500 to 3,000. They are all long-term prisoners. The bulk of the prisoners are farm labourers. All the Provinces of Egypt are represented practically in proportion to their population. The prisoners work bare-footed and march daily through the village to the quarries and back.

The water supply to the Prison is unfiltered Nile water drawn a good distance from the shore and stored in a large tank on the premises. Occasionally sedimentation is made by chemicals. No canal water is used. Although the Nile contains in certain seasons the snail *Schistosoma haematobium* carriers, yet owing to their small numbers and the large volume of water, the chances of infection are not great.

The sewage disposal, however, is primitive. Within the Prison,

it consists of bucket latrines. The buckets are removed once a day and are replaced by clean ones. The waste water runs in open cemented channels and together with the contents of the buckets is pumped away into a small sewage farm 20 acres in area. This farm is isolated in the desert at about 3 kilometres from the Prison. Some of the prisoners work on the farm. They cultivate vegetables for consumption by the prisoners after cooking. No vegetables that are eaten raw are allowed to be cultivated. (Plates I and II.)

In the quarries, square iron box latrines are provided at convenient distances. The prisoners use them and are rigorously prevented from defaecating or urinating anywhere else. At the end of the day the contents of the boxes are dumped into a trench dug in the ground. When nearly full, the trench is filled in with earth and another one is dug in another locality. Occasionally the manure, after it is kept for several months, is sold to farmers. (Plates III and IV.)

III. FIRST SURVEY OF THE PRISONERS IN 1924

The first survey in 1924 consisted of miscroscopical examination of the urine and stools of all the inmates of the Prison. They were 2,946 males; no females are kept in this Prison. The staff employed consisted of three medical men, one laboratory assistant and two attendants. One hundred individuals were dealt with every day.

The urine was passed in a conical urine glass and left to settle for at least 30 minutes. The deposit was removed by a long pipette and put on a glass slide and examined. The centrifuge was not used as it often produces more harm than good by breaking the weak egg shell of the Schistosoma ova.

The stools were examined in two different ways. A smear from the surface or from any blood or mucus, if present, is examined directly. Another sample is prepared by Khalil's floatation method in a conical erlenmeyer flask. The surface film was examined microscopically. This method is very efficient in detecting the ova of Ancylostoma, Trichostrongylus and to some extent Ascaris ova. The result of the first survey is tabulated below.

	Grand Total		Prisoners	Warders	Foreign prisoners not included
	No.	Per cent.			
Grand total	2,938	...	2,610	328	8
Free from helminths	703	24	609	94	4
Total Schistosoma infection	558	19	479	79	1
Urine :					
Schistosoma haematobium	493	17	414	79	1
Schistosoma mansoni	1	0·03	1	0	0
Stools :					
Schistosoma haematobium	9	0·3	9	0	0
Schistosoma mansoni	85	2·9	84	1	0
Ancylostoma duodenale	804	27·3	718	86	2
Ascaris lumbricoides	1,671	56·8	1,532	139	3
Enterobius vermicularis	283	7·5	277	6	0
Hymenolepis nana	14	0·5	12	2	1
Trichostrongylus sp.	144	4·9	122	22	0
Tricocephalus trichuris	225	7·7	191	34	0
Taenia saginata	3	0·1	2	1	0
Strongyloides stercoralis	19	4·9	19	0	0

It was evident from this result that the helminthic infection amongst the Prison inmates is very much lower than amongst the general population with the exception of Ascaris and Trichocephalus infections which are very high amongst the Prison inmates. It was evident also that the incidence of infection amongst the prisoners was similar to that amongst the warders showing that they were affected more or less to the same extent by this enforced mode of life. The eight foreign prisoners are not included in the statistics.

IV. GEOGRAPHICAL DISTRIBUTION OF HELMINTH INFECTION IN EGYPT

(*a*) *Schistosoma mansoni.*

An attempt was made to localise the endemic areas of infection in the case of individual parasites. The prisoners came from all Provinces of Egypt in numbers practically proportionate to their population. A striking result was found in the case of *S. mansoni* infection. All the cases came from Lower Egypt (the Delta) and none came from Upper Egypt except three who have also resided for varying periods in Lower Egypt. This peculiar localisation of *S. mansoni* infection has since been confirmed by statistics and local surveys of the helminthic infection as well as by studying the snail distribution.

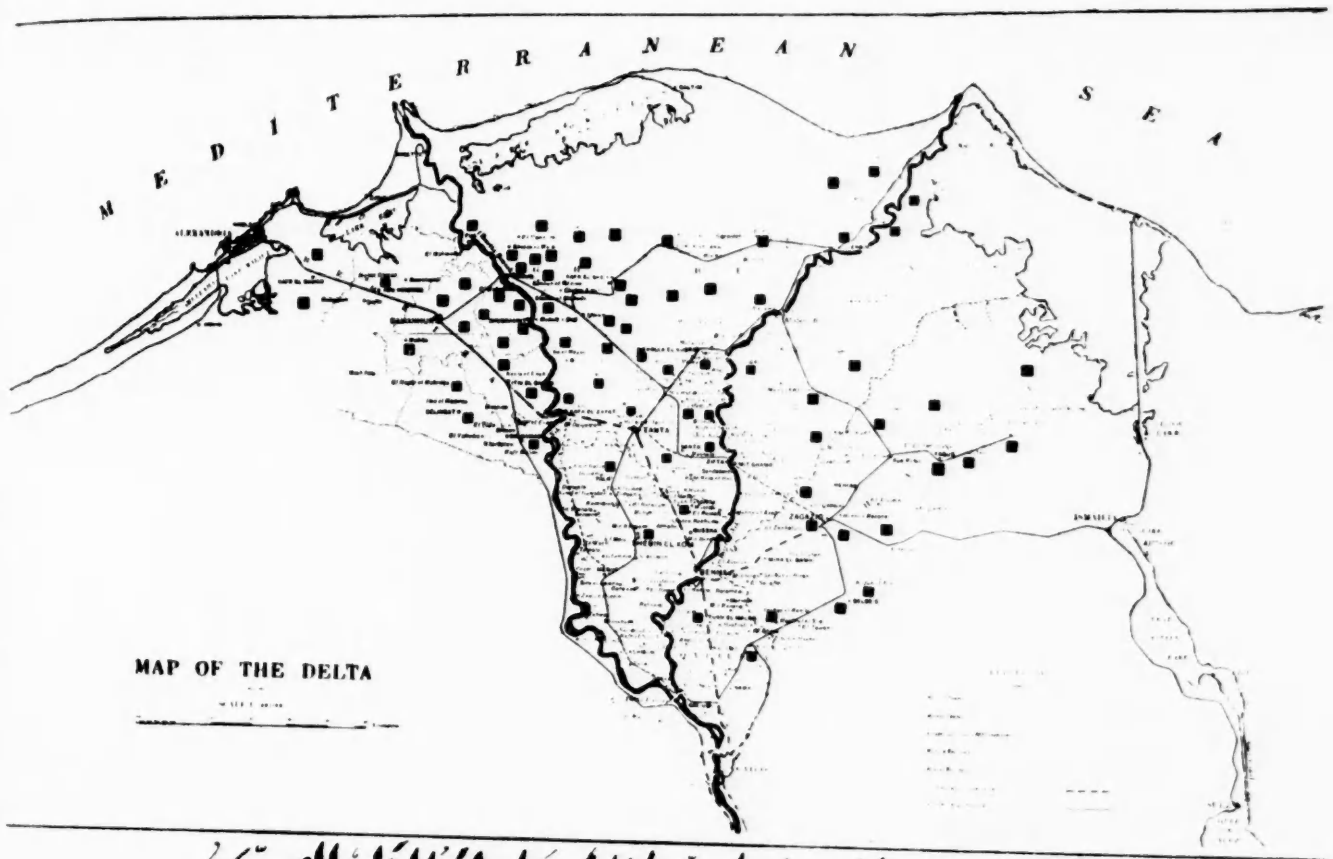
Within the Delta itself the incidence of *S. mansoni* infection seems to increase as we proceed from the South to the North. The six Provinces of the Delta are tabulated below, beginning from South to North and the incidence of *S. mansoni* amongst the prisoners is shown. The Provinces however, overlap to a certain extent. Fig. 1.

	No. examined	*Schistosoma mansoni*	
		No.	Per cent.
Qaliubia	100	2	2·0
Menufia	187	3	1·6
Sharkia	172	16	9·3
Dakahlia	100	8	8·0
Behera	159	17	10·7
Gharbia	259	33	12·7
Total Lower Egypt (prisoners)	977	79	8·0
Total Upper Egypt (prisoners)	1,449	3	0·2

The absence of *S. mansoni* infection from Upper Egypt is due to the absence of the *Planorbis boissyi* snails which are the common intermediate hosts in Egypt. The reasons for their absence are being at present studied. These snails are present in the White and Blue Niles in the Sudan and also in the Delta, but are apparently absent from the zone lying between these areas.

(*b*) *Schistosoma haematobium.*

Infection with this worm is present in all Provinces of Egypt. It is, however, as is expected, rarer in those Provinces where perennial irrigation is absent. In such Provinces the land remains dry for the most part of the year and the canals are also empty. This is illustrated by the incidence of infection in Girga and Qena Provinces. The Province of Asswan has a large area under perennial irrigation at Kom Ombo. The number of prisoners from this last Province is very small to allow definite conclusions to be made. The big cities are relatively free from infection. The inhabitants drawn from the country harbour the infection. (Fig. 2.)



انتشار عدوى بلهارسيا الأمعاء فى القطر المصرى

مسما على فحص ٣٠٠٠ نجاين فى طرة

FIG. 1. Case incidence of *S. mansoni* amongst the inmates of Tura Prison.

Provinces arranged from North to South	Irrigation	Total No. examined	*Schistosoma haematobium*		*Ancylostoma duodenale*	
			No.	Per cent.	No.	Per cent.
Delta:						
Gharbia	Perennial	259	48	18·5	60	23·2
Behera	,, ...	159	24	15·1	35	22·0
Dakahlia	,, ...	100	25	25·0	30	30·0
Sharkia	,, ...	172	34	19·8	58	33·7
Menufia	,, ...	187	27	14·4	52	27·8
Qaliubia	,, ...	100	19	19·9	42	42·01
	Total	977	177	18·1	277	28·3
Upper Egypt:						
Giza	Perennial nearly all	66	10	15·2	15	22·7
Beni Suef	,,	148	28	18·9	42	28·4
Fayoum	Perennial all ...	136	31	22·8	29	21·3
Minia	,, ...	248	51	20·6	77	31·0
Assiut	Half perennial, half basin	457	74	16·2	139	30·4
Girga	Basin	222	15	6·8	62	27·9
Qena	,, ...	155	14	9·0	43	27·6
Asswan	Basin except Kom Ombo	17	4	23·5	4	23·5
	Total	1,449	227	15·7	411	28·0
Governorates:						
Cairo	...	92	6	6·5	14	15·2
Alexandria ...	...	58	7	12·1	11	18·3
Port Said	...	28	5	17·9	5	17·9
Damietta	...	6	1	16·7	0	0
	Total	184	19	10·3	30	16·3

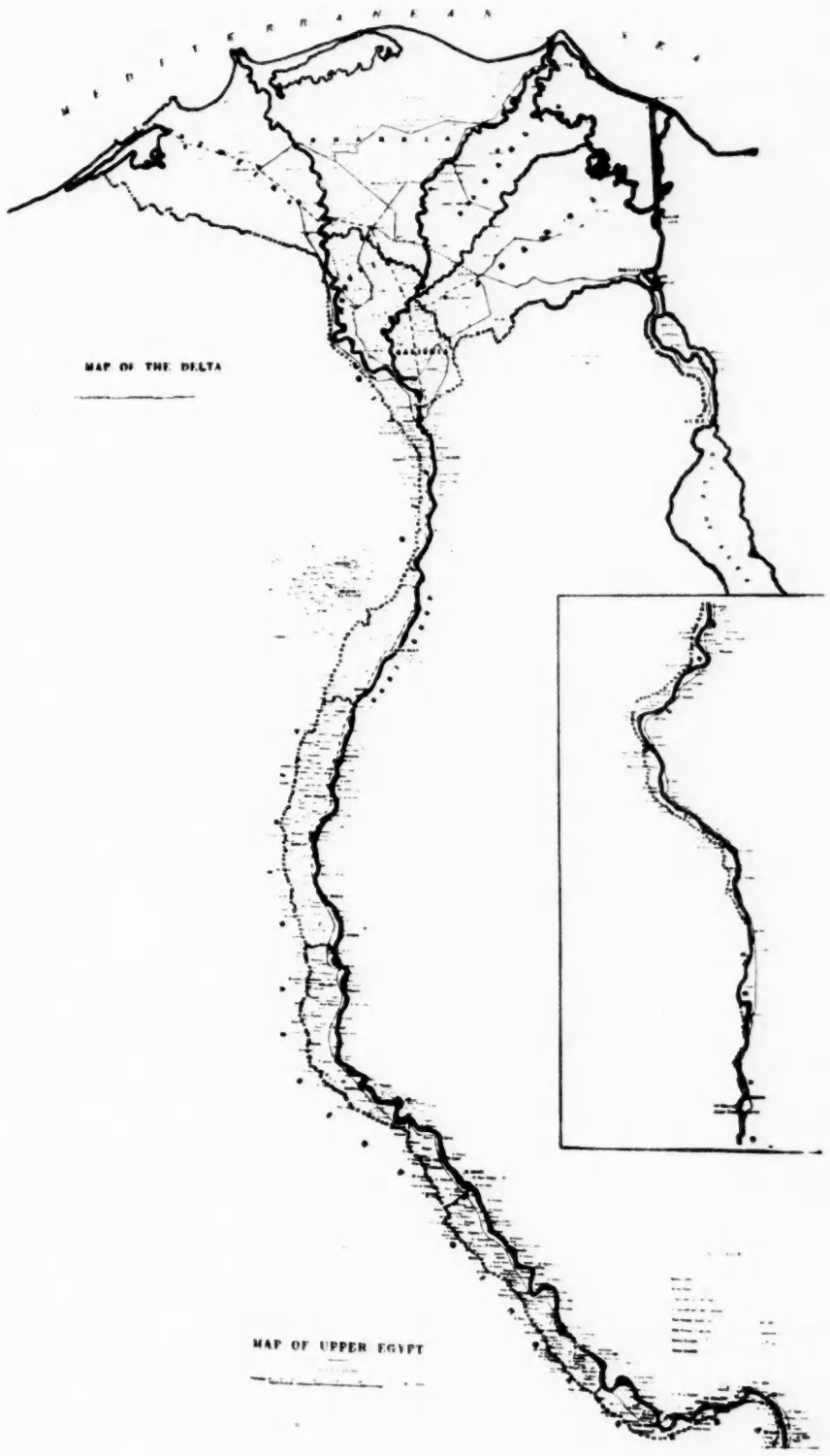


FIG. 2. Map of Egypt showing the Provinces.

(*c*) *Ancylostoma duodenale.*

This is the only species of Hookworm present in Egypt. No specimens of *Necator americanus* have yet been found naturally infecting any of the inmates of Egypt. The infection with this parasite is present in all Provinces but on the whole the infection is light. Owing to the practical absence of rain in Upper Egypt and its rare occurrence elsewhere, this factor is of minor importance in influencing Ancylostoma infection. In Egypt two factors are apparently very important in this respect :—

1. The atmospheric temperature which increases as we travel from North to South, and
2. The intensity of irrigation resulting in high subsoil water keeping the surface of the ground moist.

These two factors are practically antagonistic in Egypt, the northern Provinces enjoy perennial irrigation but are colder than the Southern Provinces which are under basin irrigation. The result is that the middle provinces around Cairo are the most intensely infected if they are under perennial irrigation.

Assiut Province is peculiar in that its northern part is under perennial irrigation while its southern part is still under basin irrigation. The contrast in the incidence of parasitic infection amongst prisoners coming from the two different sections of the Province is significant.

	No. of prisoners	*Schistosoma haematobium*		*Ancylostoma*	
		No.	Per cent.	No.	Per cent.
NORTHERN SECTION :					
Mellawi District	61	18	29·5	35	57·4
Deirut District	36	15	41·7	22	61·1
Manfalout District	8	2	25·0	5	62·5
Abnoub District	22	3	13·6	17	77·3
Total ...	127	38	30·0	79	62·2
SOUTHERN SECTION :					
Assiut District	15	2	13·3	8	53·3
Abu Tig District	8	3	37·5	3	37·5
Badari District	25	1	40·0	13	52·0
Total ...	48	6	12·5	24	50·0

(*d*) *Ascaris lumbricoides.*

Ascaris infection amongst the prisoners and warders is very high. The percentage of infection is slightly lower amongst the warders, who have got more liberty in the choice of their food. The prisoners and warders from the different parts of Egypt are practically infected to the same extent contrary to the condition with *S. mansoni, S. haematobium* and *Ancylostoma.* This hints at a common factor affecting all the inhabitants of the Prison, as the infection amongst the general population is not uniformly so high. This subject will be dealt with later.

	No.	*Schistosoma haematobium*		*Ancylostoma*		*Ascaris lumbricoides*	
		No.	Per cent.	No.	Per cent.	No.	Per cent.
Prisoners	2,610	414	15·8	718	27·3	1,532	58·7
Delta (Lower Egypt) ...	977	177	18·1	277	28·3	593	60·7
Upper Egypt	1,449	227	15·7	411	28·0	821	56·6
Governorates	184	19	10·3	30	16·3	118	64·1
Guards and Staff ...	328	79	24·1	86	26·2	139	42·3
Delta (Lower Egypt) ...	129	37	28·7	40	31·0	52	40·3
Upper Egypt	168	39	23·2	42	25·0	72	42·8
Governorates	31	3	9·7	4	13·0	15	48·3

V. CONTRAST BETWEEN THE INMATES OF TURA PRISON AND THE GENERAL POPULATION AS REGARDS THE INCIDENCE OF PARASITIC INFECTION

Judging from the general results of the survey, it is apparent that the incidence of parasitic infection in Tura Prison is different from that occurring in the peasant population. Schistosoma and Ancylostoma infections are less frequent while Ascaris and Trichocephalus infections are more common. In order to confirm this, the prisoners were divided into groups according to the year in which they entered the Prison. It is evident from the

accompanying table and graphs (Fig. 3) that Schistosoma infection affects a higher percentage of the newly admitted prisoners and they gradually become free from it in proportion to the period they pass in the Prison. The same is true about Ancylostoma infection. Ascaris infection on the other hand is very rife amongst the prisoners, more so than amongst the general population and it seems to be not affected by the length of stay in the Prison. The same is more or less true of Trichocephalus infection. The mode of life of the prisoners is different from that of the peasant population from whom the majority are withdrawn in the following respects :—

1. The prisoners work in quarries instead of agriculture.
2. They drink and bathe with clarified Nile water instead of unfiltered canal water rich in Molluscan fauna.
3. They are not allowed to pollute the soil with their excreta while in the villages pollution of the soil is the rule.

In both conditions they are lightly clad and work bare-footed.

Date of imprisonment	Total No.	*Schistosoma haematobium*		*Ancylostoma*		*Ascaris*		*Trichocephalus*	
		No.	Per cent.	No.	Per cent.	No.	Per cent.	No.	Per cent.
1923	248	76	30·6	167	67·3	188	75·8	19	7·7
1922	338	79	23·3	161	47·5	209	61·7	22	5·4
1921	259	62	23·9	109	42·1	124	47·9	19	7·5
1920	190	35	18·9	59	31·0	110	57·9	15	7·9
1919	219	32	14·6	43	19·7	132	60·3	29	13·2
1918	144	23	16·1	20	14·0	87	60·9	13	9·1
1917	130	22	16·7	14	10·8	72	55·4	4	3·1
1916	170	17	10·0	15	8·8	92	54·1	12	7·1
1915	139	9	6·5	7	5·0	83	59·3	7	5·0
1914	95	12	12·6	9	9·0	60	63·0	3	3·1
1913	99	5	5·0	12	12·0	52	52·0	3	3·0
1912	78	2	2·6	5	6·5	39	50·7	5	6·5
1901–11	261	12	4·6	39	14·9	159	60·5	25	9·6

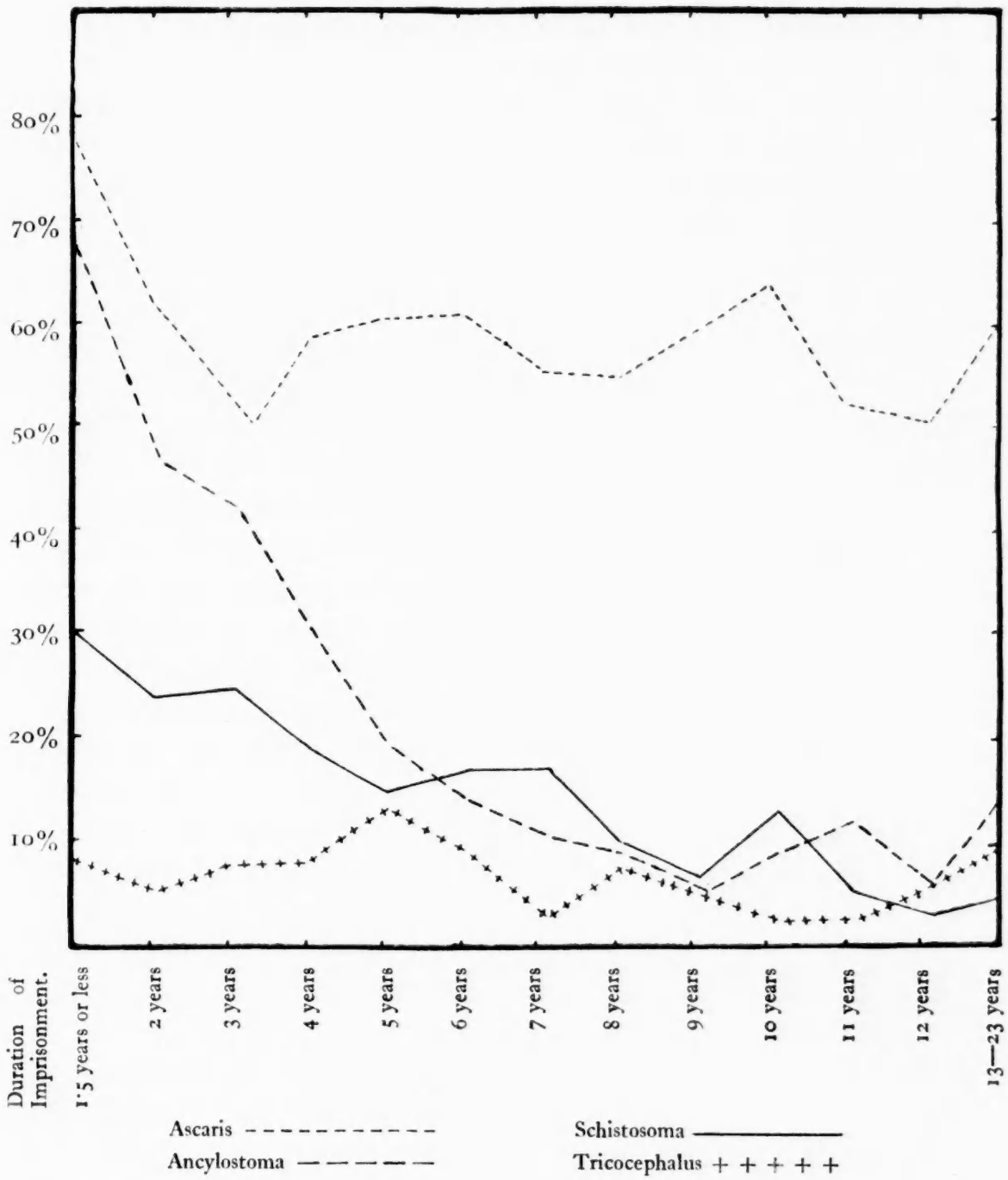


FIG. 3. Effect of the duration of Imprisonment upon the Incidence of Parasitic Infection amongst the inmates of Tura Prison.

VI. THE EFFECT OF SANITATION ON HELMINTHIC INFECTION

(*a*) *The Water Supply and Schistosoma Infection.*

Schistosoma infection takes place only through the medium of water containing the cercariae. Tura Prison abuts on the Nile on one side and a local canal " Khashab Canal " on the other. Both Streams are known to be sources of Bilharzia infection in varying degrees. The Nile is known to contain Bullinus snails during the early summer, but the chance of infection from drinking Nile water taken a certain distance from the shore is not great. Khashab Canal, on the other hand, is the source of infection to 75 per cent. of the population of the villages in the immediate neighbourhood of the Prison. The contrast as regards Schistosoma infection amongst the people living on either side of the Prison wall is astounding.

The cause of this is evident. The prisoners have a water supply for drinking purposes from the Nile, while the inhabitants very often draw their water from the canal. The prisoners are not engaged in agriculture, while the inhabitants are. This necessitates the frequent immersion of the limbs in the water. Schistosoma infection is principally a skin infection. The prisoners do not contract new infection and they gradually get rid of their old Bilharzia infection by the natural process of the adult worms dying out.

Systematic examination and treatment for Bilharzia infection was not in force in the Prison. The graph for Schistosoma infection indicates that natural cures are fairly common if the chances of re-infection are avoided. It was a general belief amongst practitioners and wealthy patients in Egypt before the specific treatment of Bilharziasis was discovered that a stay in Europe for several years will result in curing Bilharziasis. They attributed this to the effect of the European mineral waters and climate. It is, however, a natural condition.

(*b*) *Soil Pollution and Ancylostoma Infection.*

Soil pollution is strictly forbidden in the Prison and in the quarries by enforcing the system of sanitation described before. In the villages lying in the neighbourhood of the Prison soil pollution is widespread. In the village of Maasara, lying about three kilometres

from the Prison, 88 per cent. of the inhabitants are infected with Ancylostoma. This is the highest record yet found in any Egyptian village. The prisoners in the absence of any source of re-infection gradually get rid of Ancylostoma infection. The effectiveness of this simple method of conservancy lies in its strict supervision. Thus it is demonstrated that even with very simple methods great success may be attained in controlling Ancylostomiasis. A similar method is carried out throughout the towns of the Sudan, including Khartoum itself. Buckets are used and removed systematically by a special gang. They are taken outside the town where the contents are dumped, buried or burnt. The buckets are disinfected, cleaned and returned for further use. The efficiency with which this method is worked is evident from the relative unimportance of Ancylostoma infection in the Sudan and the effective control of the fly nuisance.

The reduction of Ancylostoma infection from 67.3 per cent. amongst the newly admitted prisoners to 8 to 10 per cent. amongst those that remained ten or more years in prison is an evidence of the value of conservancy as a permanent means for the control of Ancylostomiasis. In the meantime, such a result reflects a good deal of credit on the sanitary squad of Tura Prison.

The sewage, after lying in the pit for about three months, is practically safe to use as fertiliser as far as Ancylostoma infection is concerned. The ova are known to persist more in cooler weather. In Egypt where the temperature is fairly high, three months may be regarded as a safe limit.

(*c*) *Contamination of Food and Ascaris Infection.*

The principal source of Ascaris infection is still being disputed. The observations made at Tura Prison and in other localities in Egypt leave no doubt that contaminated food is the principal vehicle for Ascaris infection, especially contaminated vegetables.

The water supply of the Prison is not the cause, as such a heavy infection does not exist in the neighbouring villages dependent on the Nile and Canal for their water. Soil pollution does not exist and the effect of this on Ancylostoma infection was demonstrated. The source of the high Ascaris infection remained, however, a puzzle until it was found that to the Prison is annexed a small sewage farm at a distance of three kilometres, where the sewage

from the Prison is used for irrigation and for fertilizing the sandy soil. The farm is about twenty acres in area, isolated in the desert, having no water supply other than the sewage of the Prison. Prisoners cultivate it. Vegetables are grown for the consumption of the prisoners, but care is taken not to grow vegetables that are eaten raw. A visit to the farm revealed that the sewage is kept in large storage tanks until it is needed. Vegetables for the kitchen are grown in abundance.

The kitchen was then visited and a large heap of vegetables was found there and some of the prisoners were busy preparing it. A small heap of earth, including the roots of the vegetables accumulated. On examination of this earth by direct microscopic examination of smears and by the salt floatation method, fully developed ova of Ascaris were found. It was evident that the hands of all prisoners engaged in the kitchen were contaminated and in turn they contaminate the food when it is being distributed after cooking.

The same conclusion was arrived at as a result of examining two other localities in Egypt.

The first was the town of Port Said. It has a filtered water supply above suspicion. The city is provided with a sewage carriage system. No endemic cases of Bilharziasis or Ancylostomiasis are found, as the town is surrounded by either salt water or desert and there is practically no agriculture. An Anthelminthic Hospital, established in the town in 1924, revealed the comparative rarity of Ancylostoma and Schistosoma infection amongst the inhabitants themselves and a high incidence of Ascaris infection—73 per cent. amongst 3,181 cases examined. Dr. Stiven, Principal Medical Officer of Port Said Government Hospital, ascribed this to pig breeding. This, however, is a limited affair, as the majority of the inhabitants are Mohammedans to whom the pig is prohibited as food and is an undesirable animal. Moreover, the pig parasite is not transferable to man and vice versa. Port Said, however, has a sewage farm. Although the tenant of the farm is warned against growing vegetables that are eaten raw, yet the high prices of such articles at Port Said proved to be a temptation difficult to resist, with the result that these orders are seldom obeyed.

On the other hand, a survey of the Helminthic infections at Kom Ombo in Asswan Province revealed a very low percentage of Ascaris infection.

The incidence of Schistosoma infection was 82 per cent., pointing to free contamination of the water courses. Ancylostoma infection was 24 per cent., pointing to a fair degree of soil pollution. The sanitary condition of the villages in the area is deplorable. No provision is made for water supply. The water is collected from any neighbouring canal open to contamination by man and beast. Practically no house in the whole group of villages forming the estate is provided with a latrine. The reason for the low Ascaris infection remained obscure until it was revealed that human manure is never used as a fertilizer in the fields in the whole area. The estate belongs to a company who utilizes the earth of neighbouring hills as manure. By means of an intricate system of light railways, the manure is transported wherever it is wanted.

It is thus evident from the three different localities described that the only factor of importance in Ascaris infection is contamination of food, particularly vegetables, by human manure. In Tura Prison, owing to the neglect of this factor only all other parasites are efficiently controlled while Ascaris and Trichocephalus infections flourished thus emphasising this factor.

VII. TREATMENT OF ANCYLOSTOMIASIS AND SUBSEQUENT SURVEYS

5 c.c. of carbon tetrachloride were administered to every one of the prisoners in May, 1924, all on the same day. Out of the 2,618 cases thus treated, only 52 complained of any symptoms as a result of the treatment. In many cases it was possibly malingering, 47 complained of colic, 33 vomited, 46 of giddiness, 26 of pain in the throat, 26 of pain in the anus, 36 of constipation. It is evident from the above figures that each individual complained of many or all these symptoms at the same time.

Re-examination was made after one year for the detection of Ancylostoma infection. Of the 2,618 prisoners present in 1924, 2,276 remained in 1925, while the rest were discharged or transferred to other localities. The result was as follows :—

Total number re-examined	2,276
Ancylostoma infection in them in 1924	431 (19%)
Ancylostoma infection in them in 1925	154 (6·7%)
Persistent Ancylostoma infection in old cases	119 (5·2%)
Newly infected individuals	35 (1·5%)

Re-examination in 1926, after the lapse of another year, revealed the following results. No treatment was administered during the interval.

Number of prisoners remaining in 1926	1,612
Ancylostoma infection in them in 1924	375 (23·3%)
Ancylostoma infection in them in 1925	84 (5·2%)
Ancylostoma infection in them in 1926	48 (3·0%)
Persistent Ancylostoma infection	36 (2·2%)
Stools positive since 1924	24 (1·5%)
Stools positive in 1924, negative in 1925	12 (0·7%)
Newly infected individuals	11 (0·7%)

Re-examination in 1928, after the lapse of another two years, revealed the following results. No treatment was administered during the interval.

Number of prisoners remaining in 1928	945
Ancylostoma infection in them in 1924	183 (19·4%)
Ancylostoma infection in them in 1925	47 (5·0%)
Ancylostoma infection in them in 1926	17 (1·8%)
Ancylostoma infection in them in 1928	5 (0·5%)
Persistent Ancylostoma infection (positive in 1924, negative in 1925 and 1926)	1 (0·1%)
Newly infected individuals	4 (0·4%)

The small percentage of re-infection with Ancylostomiasis probably represents individuals exposed to infection on the sewage farm or from being transferred to other prisons where the conditions are more favourable for infection with Ancylostoma. This transfer is commonly made for need of working hands. The small number of cases in whose stools ova were found in one year and not in another probably represented very light infection that occasionally remains undetected.

VIII. SUMMARY

1. An examination for Helminthic infections of the prisoners in the Principal Convict Prison of Egypt revealed a peculiar localisation of *Schistosomiasis mansoni* in Lower Egypt. This was confirmed by surveys in Upper Egypt for this Helminthic infection and for the snail carriers where both were found to be absent.

2. The incidence of Schistosomiasis and Ancylostomiasis amongst the prisoners was lower than amongst the general peasant population from whom they were principally drawn. This was found to be due in the case of Schistosomiasis to the absence of re-infection,

owing to a clarified water supply and gradual elimination of the parasites by time. In the case of Ancylostomiasis this was due to practical restriction of soil pollution preventing re-infection and gradual elimination of the parasites by time.

3. The incidence of Ascariasis and Trichocephalus infection was more than amongst the general peasant population. This was found to be due to the presence of a small sewage farm utilizing the sewage of the Prison for irrigation and fertilization of the soil. The vegetables grown are contaminated with the ripe ova which develop favourably in the warm shady and moist soil of the farm. Although these vegetables are eaten after cooking, yet the contaminated hands of the kitchen staff and utensils afford ample chances for infection.

4. Ancylostoma infection is practically eliminated in four years after a single treatment provided that proper sanitation guards against re-infection.

5. It is evident from the survey that the principal route of Schistosoma infection is through infected water by way of the skin ; of Ancylostoma infection is through contaminated soil by way of the skin ; and of Ascaris infection is through contaminated food.

6. The pail system of sewage disposal is both very simple and efficient in controlling Helminth infection if it is efficiently supervised. The system is in force in many towns of the Anglo-Egyptian Sudan with success. Other elaborate methods of sewage disposal are not necessarily efficient in controlling helminths. The final effluent should never be allowed into a stream or canal, the water of which is used for household purposes, unless it is proved microscopically to be free from ova and larvae.

7. In Egypt the sewage stored in a trench can be considered safe for use on the fields after three months.

8. Treatment with carbon tetrachloride was administered in 5 c.c. doses to 2,618 cases without any grave accident. One treatment was sufficient to cure 64.3 per cent.

IX. ACKNOWLEDGMENTS

This investigation was carried out by the kind permission of H. E. Dr. Mohammed Shahin Pacha, Under-Secretary of State for Public Health, who took great interest in pursuing its development.

It gives me pleasure to thank H. E. Tewfik Pacha Abdullah, Director General of the Prisons Department, for the facilities offered. Dr. M. Arafa, Medical Officer of Tura Prison, was always helpful in many ways.

Thanks are due to the Medical Officers and Laboratory Assistants who carried out the microscopical examinations—Drs. M. Abaza, A. H. Rabie, M. Nazmi, and the Laboratory Assistants Ali Hassan, Hadari and Barakat.

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PLATE I

EXPLANATION OF PLATE I

Fig. 1. Two pail closets.

Fig. 2. A series of pail closets.

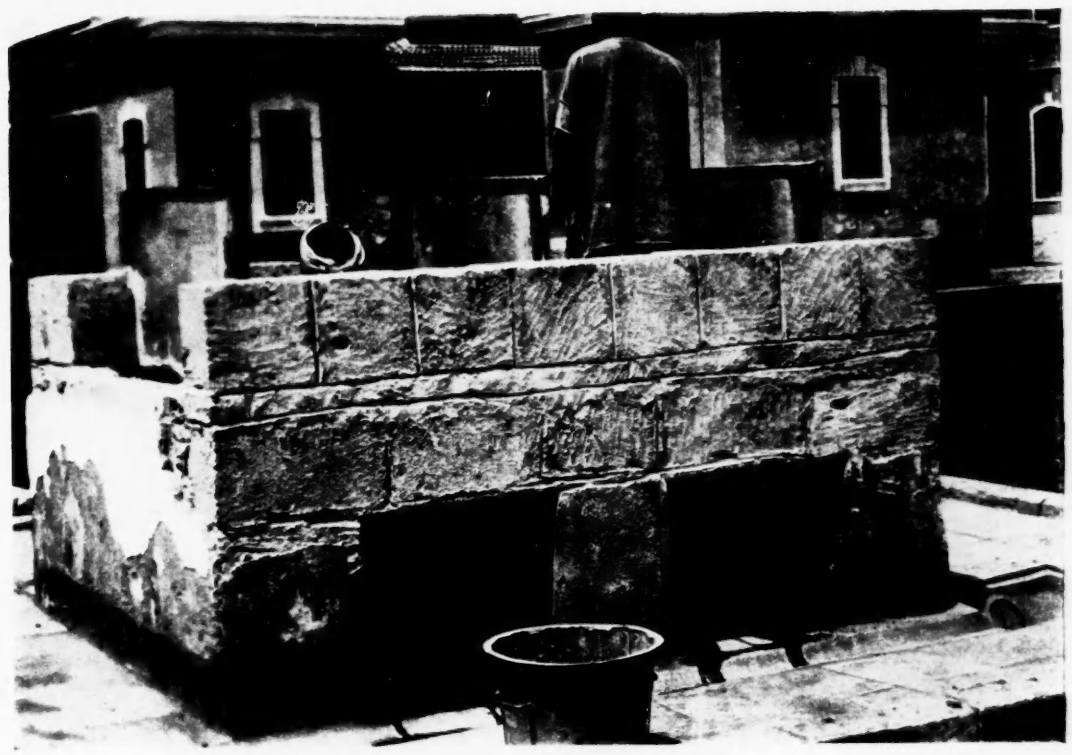

FIG. 1

FIG. 2

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EXPLANATION OF PLATE II

Fig. 1. Cemented sewage channel inside the Prison.

Fig. 2. Reservoirs in sewage farm.

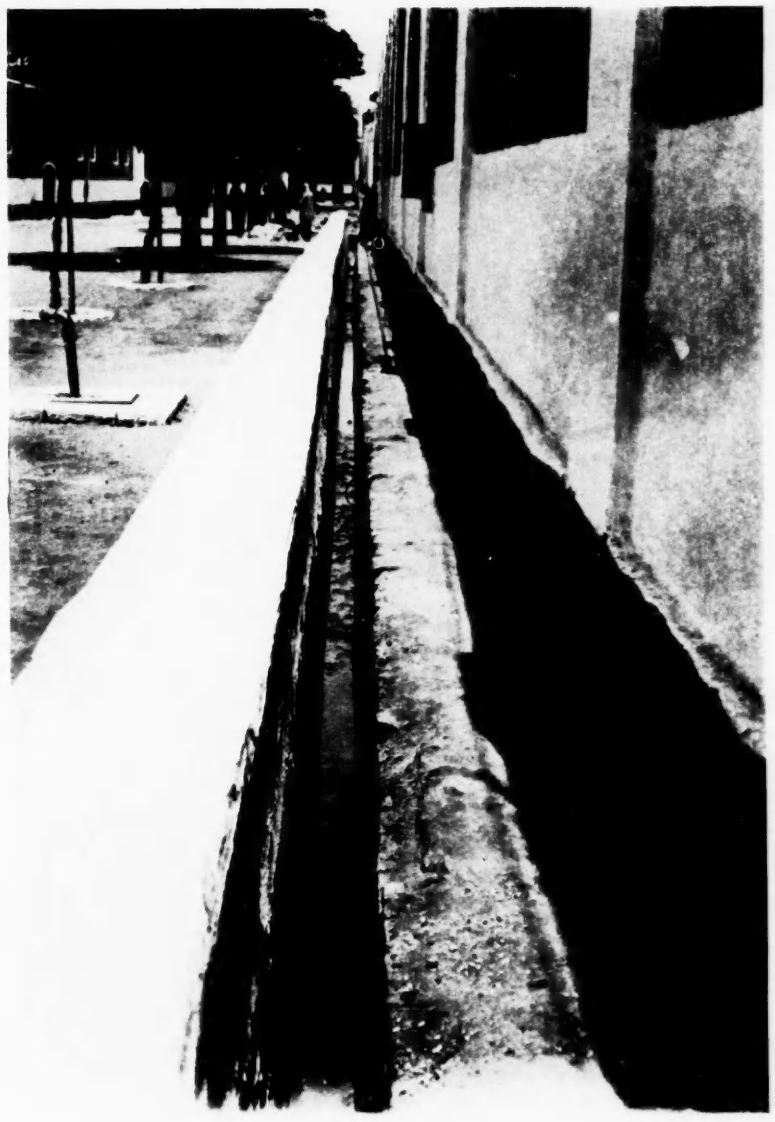

FIG. 1

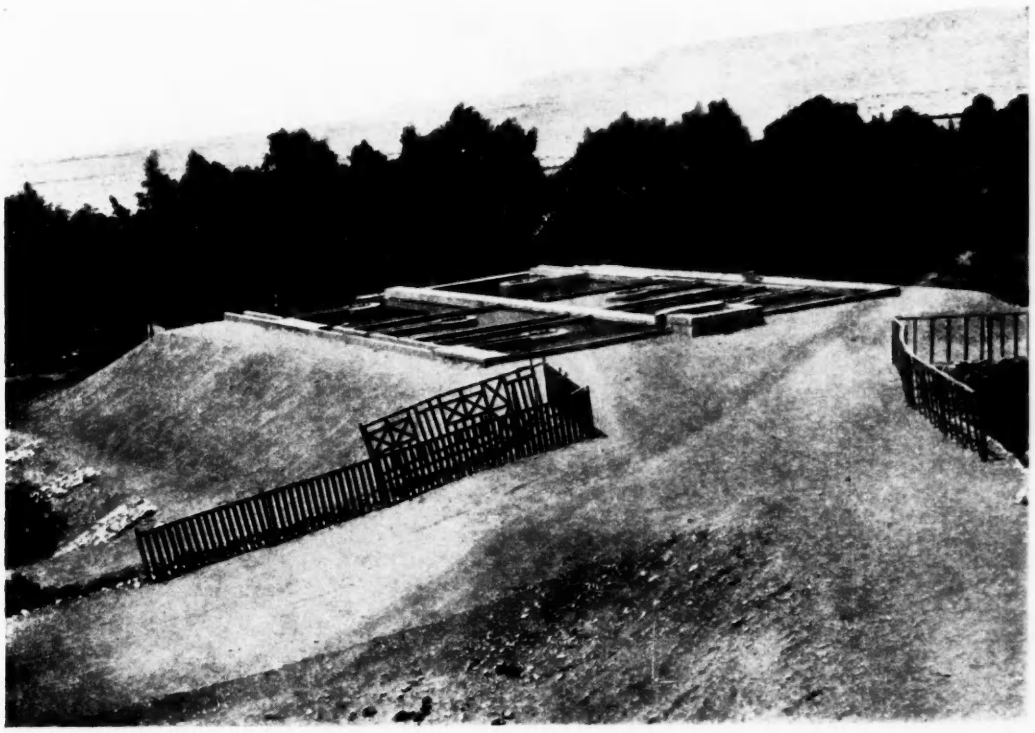

FIG. 2

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EXPLANATION OF PLATE III

Fig. 1. Iron box latrines in the quarries.

Fig. 2. Iron box latrine with a guard posing on it.

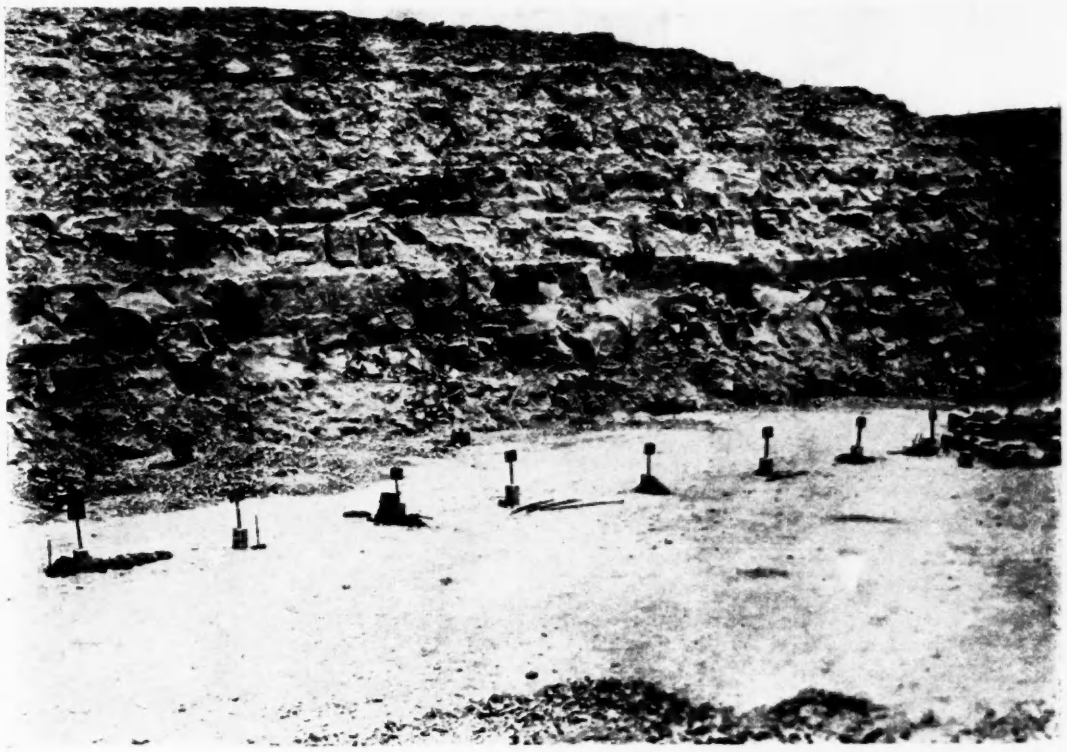

FIG. 1

FIG. 2

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EXPLANATION OF PLATE IV

Trench for dumping contents of box latrines.

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A FURTHER NOTE ON SOME CASES OF RHODESIAN SLEEPING SICKNESS TREATED AT IKOMA, TANGANYIKA TERRITORY, IN 1926 AND 1927, INCLUDING THOSE UNDER THE CARE OF PROFESSOR F. K. KLEINE

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(*Received for publication* 10 *November*, 1930)

INTRODUCTION

During a short visit to Ikoma, in September, 1930, I made enquiries about cases of sleeping sickness treated there in 1926 and 1927, by Professor Kleine and myself. Details of thirty-nine of these cases were published in a previous paper (Corson, 1928), and the first thirty-seven of these had been under Professor Kleine's care. A further twenty-seven cases were treated up to the ninth of October, 1927, soon after which I left Ikoma. This area is comparatively favourable to the task of following up cases. It is somewhat circumscribed, most of the patients coming from villages situated within a radius of about 12 miles from the sleeping sickness hospital at Ikoma, and there is only one hospital or treatment centre in the area. The village people seem to know all the news of the place and, when one has lived for a year or two there, it is not difficult to get reliable accounts from various sources such as hospital staff, patients, patients' relatives, headmen and chiefs. Unfortunately, weather conditions restricted my stay to eight days, so that I was unable to see as many survivors as I wished to see. Since Dr. Park Noble's departure, in July, 1928, the sleeping sickness hospital at Ikoma has been in charge of a succession of members of the subordinate medical staff, viz., Indian and Goan Sub-assistant Surgeons and

TABLE

Case No.	Date when diagnosed microscopically	Parasitic blood relapse	Result	Notes
21	15 May, 1926	+	Dead.	...
22	19 ,,	+	Dead.	...
23	19 ,,	−	Reported alive.	...
25	21 September, 1926	+	Reported alive.	...
26	23 ,,	+	Dead.	...
34	25 ,,	+	Dead.	...
35	26 ,,	−	Dead.	...
37	26 ,,	−	Dead.	...
39	29 ,,	+	Dead.	...
40	23 ,,	+	Dead.	...
41	2 October, 1926	+	Dead	...
43	20 ,,	+	Dead.	...
44	23 ,,	+	Dead.	...
45	29 ,,	−	Reported alive.	...
46	30 ,,	+	Seen alive.	...
47	5 November, 1926	+	Dead.	...
49	10 ,,	−	Reported alive.	...
51	16 ,,	−	Seen alive.	C.S.F. normal.
52	9 ,,	+	Dead.	...
53	20 ,,	+	Dead.	...
54	28 ,,	+	Dead.	...
57	5 December, 1926	+	Seen alive.	Ill.
58	5 ,,	+	Dead.	...
59	8 ,,	+	Dead.	...
60	12 ,,	+	Dead.	...
61	15 ,,	−	Seen alive.	C.S.F. normal.
62	16 ,,	−	Dead.	...
64	7 January, 1927	−	Dead.	...
66	14 ,,	+	Dead.	...
67	28 ,,	−	Seen alive.	...
68	8 February, 1927	+	Dead.	...
70	11 ,,	−	Reported alive.	...
71	12 ,,	−	Seen alive.	...
73	1 March, 1927	−	Dead.	...
74	17 ,,	−	Reported alive.	...
75	29 ,,	+	Dead.	...
76	6 April, 1927	−	Dead.	...
77	28 ,,	+	Reported alive.	...
78	17 May, 1927	−	Seen alive.	C.S.F. normal.
79	20 ,,	−	Reported alive.	...
80	25 ,,	−	Dead.	...
81	4 June, 1927	−	Seen alive.	...
82	21 ,,	−	Seen alive.	...
83	25 ,,	−	Reported alive.	...
84	28 ,,	−	Reported alive.	...
86	4 July, 1927	−	Seen alive.	...
87	25 ,	+	Seen alive.	...
88	11 August, 1927	−	Seen alive.	...
89	16 ,,	−	Dead.	...
90	21 ,,	−	Dead.	...
91	30 ,,	−	Seen alive.	...
92	18 September, 1927	−	Dead.	...
93	20 ,,	+	Dead.	...
94	21 ,,	−	Seen alive.	...
95	23 ,,	−	Dead.	Cause doubtful.
96	23 ,,	−	Seen alive.	...
97	26 ,,	+	Dead.	...
98	28 ,,	−	Reported alive.	...
99	2 October, 1927	+	Dead.	...
100	2 ,,	+	Dead.	...
101	2 ,,	−	Seen alive.	C.S.F. normal.
102	2 ,,	−	Seen alive.	...
103	5 ,,	−	Seen alive.	...
104	9 ,,	−	Dead.	...

Compounders. The records, therefore, could not be expected to have the same value as if a Medical Officer had been there but, in my opinion, they are substantially accurate and have been kept in considerable detail in regard to the administration of the trypanocidal drugs, Bayer 205, and Tryparsamide. Because standard courses of treatment have been laid down for the use of the subordinate medical staff, the patients who have been treated there during the last two years have received at least ample quantities of these drugs, as the hospital records show. Failure to recover has, therefore, not been due in any degree to inadequate drug treatment, so far as we are at present able to judge.

Results. The results of the sixty-four cases referred to are shown in the Table. Twenty-nine (45·3 per cent.) were reported to be alive, and I saw eighteen. All were said to be well except two, viz., Case 57 (whom I saw) and Case 77 who was said to be very ill and insane in his village, and whom I had not time to visit, though asked to do so. The figures are not so good with regard to the thirty-seven patients, referred to above, who came under Professor Kleine's care in the period from September, 1926, to May, 1927. Twelve (32·4 per cent.) were believed to be alive and I saw six. Of the other twenty-seven patients, seventeen (63 per cent.), of whom I saw twelve, were said to be alive. The difference in the figures is due to the fact that a greater proportion of early cases came during the later months of the year 1927, and is not related in any way to treatment. The reason, in my opinion, is that by about the middle of 1927 the people had got to know us and to recognise the quick temporary or permanent recovery after treatment with Bayer 205, and had not yet learnt that so many relapse and die.

Parasitic blood relapses. A study of the details of these cases shows that when a parasitic blood relapse has occurred, the prognosis is very unfavourable. Very few recovered in spite of thorough and prolonged treatment. These few cases are of some interest. In the list shown in the Table there are five cases that were still alive and had had a parasitic blood relapse, viz., Cases 25, 46, 57, 77 and 87. Of these, two, viz., Cases 57 and 77, were ill and the prognosis is unfavourable. Case 57 is the sole survivor of four cases in the family, viz., Case 58 (his wife), Case 62 (his wife's mother), and Case 66 (his son). He has had many parasitic blood relapses at short

intervals and, consequently, many courses of treatment with drugs. I did not see Case 25, but saw the remaining two cases. Both patients refused lumbar puncture, but they appeared to be in good health. Case 46, Nyamurri, had had no treatment since May, 1928. His recovery is remarkable because, although he had no parasitic blood relapse after December, 1926, lumbar puncture in November, 1927, showed a cell count of 350 per cubic millimetre. Were it not for this, since his relapse occurred whilst still in hospital and reinfection could be excluded almost with certainty, one might be inclined to regard it as similar to those cases of transitory relapse mentioned by Tanon and Jamot (1924), though I have not seen such cases in Rhodesian sleeping sickness. It appears to be a genuine case of the recovery of a patient with an infected central nervous system. This is not certain, however, as trypanosomes were not found in the cerebro-spinal fluid.

The other case may be briefly outlined as follows :—

Case 87, Takama, son of Chirenye, aged 20, admitted on 25th July, 1927, with a history of illness of four months' duration, and complaining of headache and pains in the limbs. He was able to walk well and was not wasted. On the day of admission, his blood showed malarial parasites, but no trypanosomes which were found in rather scanty numbers on July 31st. Two guinea-pigs were inoculated with his blood on August 1st, but only one became infected. It showed posterior nuclear forms and died on the 25th day, but it was a young animal.

Treatment. After a dose of B.R.68, to test the new drug, on August 1st, trypanosomes reappeared within forty-eight hours, and he then received a course of six injections of 1 gramme doses of Bayer 205. He was allowed on leave on the 7th September, and instructed to return on the 27th, for a course of Tryparsamide injections. He failed to appear and on the 18th November, 1927, he was carried to hospital in a semi-conscious state, with numerous trypanosomes in the blood. He received 1 gramme doses of Bayer 205 on November 18th, 20th, 22nd and 24th, and on the 22nd November he was able to stagger and could talk. There were marked tremors. He received 3 gramme doses of Tryparsamide on December 1st, 8th, 15th and 22nd, and again on January 7th, February 2nd and 15th, 1928, and left the hospital. On June 17th, 1928, he visited the hospital and was well and got no treatment. Since then there is no note about him until I saw him in September, 1930, when he was apparently quite well. In this case there is a fairly probable history of infection while visiting the neighbouring district of Maswa. One can only speculate on the possibility of its being a case of a weak strain of trypanosome in a somewhat resistant patient or that the apparent blood relapse was really a reinfection.

Cases 43 and 67. These two cases may be referred to because Professor Kleine (1928) gave them as examples of the action of Bayer 205 on the infected central nervous system. Their later history is as follows :—

Case 43, Merana, son of Sehrri. On November 7th, 1928, returned to hospital, weak, wasted and complaining of headache and pains in the limbs. Trypanosomes and malaria parasites were present in the blood. He received 1 gramme doses of Bayer 205 on November 8th, 10th and 15th, and 2 gramme doses of Tryparsamide on November 29th and December 6th, 13th and 20th. On April 8th, 1929, the blood was negative. He received doses of $2\frac{1}{2}$ grammes of Tryparsamide on April 9th, 16th, 23rd and 30th, and May 7th, 14th, 21st and 28th, a total of 20 grammes. On May 30th, 1929, lumbar puncture was performed and no trypanosomes were seen, but no cell count was made. He was given leave for two months, but was not thought to be very well. I saw his father in September, 1930, who told me that Merana had again become ill but, instead of going to hospital, he had visited a native medicine-man and had died about the middle of 1930.

Case 67, Nyambura, daughter of Kisseko. She had no treatment after June, 1928, but is noted as being in good health on September 29th and October 12th, 1928, and on March 7th, 1929. I saw her at Ikoma, in September, 1930, and found her in apparently quite good health. In this case there had been no parasitic blood relapse and no lumbar puncture had been made.

SUMMARY

1. Of sixty-four patients who received thorough treatment, twenty-nine were reported to be alive about three or more years later. Twenty-seven, or 42 per cent., may be regarded as recovered. This high figure is due to the number of early cases that came for treatment, especially during the later months of the year 1927.

2. There is no indication, from a study of these cases, that any trypanocidal drug, except Bayer 205, can cure Rhodesian sleeping sickness.

3. When a parasitic blood relapse has occurred, the prognosis is very unfavourable. In all except a few cases, no form of treatment

or combination of trypanocidal drugs seems to lead to cure. Nervous symptoms are sometimes relieved by Tryparsamide when Bayer 205 has failed and the converse occurs, but the relief is only temporary.

4. One case, viz., No. 46, appears to be one of recovery of a patient with an infected central nervous system.

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NEUTRALIZATION TESTS WITH IMMUNE YELLOW FEVER SERA AND A STRAIN OF YELLOW FEVER VIRUS ADAPTED TO MICE

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(*Received for publication* 21 *November*, 1930)

INTRODUCTION

In a previous paper (1930) evidence has been presented that white mice are susceptible to the virus of yellow fever when injected intracerebrally; that the virus could be propagated indefinitely by mouse brain to brain passage; that yellow fever virus adapted to mice became attenuated for rhesus monkeys; and that the serum from a monkey immune to the virus of yellow fever as well as the sera of two human convalescents showed a definite neutralizing action on the mouse virus.

In this paper neutralization tests with thirteen human immune yellow fever sera are described. For these sera I am indebted to the courtesy of Dr. W. A. Sawyer of the Rockefeller Foundation. All the sera, according to information supplied by Dr. Sawyer, had been tested against African yellow fever virus in monkeys and all protected, although in most instances only one monkey was used in the experiment.

EXPERIMENT I

In this experiment, ten sera from yellow fever patients and one normal serum were tested. The virus was prepared by breaking up thoroughly in 9 c.c. of saline the complete brain of a mouse dying on the fifth day after an intracerebral injection of passage virus. The suspension of brain was centrifuged for thirty minutes at low speed to remove the larger particles of brain. This con-

centration we are accustomed to call a 1 : 20 dilution of virus, although the degree of dilution is probably considerably higher as there is a large portion of the virus in the particles of brain which are removed by centrifugation. Equal parts of virus and sera were well mixed and the mixture injected intracerebrally either immediately or after standing at room temperature for five to fifteen minutes. Six to eight mice were used for each mixture. The history of the sera employed in the test, as well as the fate of the mice receiving the mixtures, are shown in Table I.

Of eight mice which received the mixture of normal serum and virus, all died from the sixth to the ninth day after injection. The survival of mice for a longer period than nine days and especially those which survived twenty-one days after injection when the experiment was terminated was considered to indicate protection to a greater or less extent by the serum under investigation. Mice which died from the first to the fourth day are of no value in the interpretation of results, as they in all probability died of some intercurrent disease. Even with the most potent undiluted passage virus, death very seldom took place on the fourth day.

Examination of the table shows that under the conditions of the experiment all the sera with the exception of one (No. 261) protected one or more mice for at least twenty-one days. In the results obtained it was considered that all the sera showed definite protection except No. 261, which was interpreted as doubtful. This serum, as well as two others (Nos. 442 and 152), were retested in later experiments.

EXPERIMENT II

Thinking that by the use of a more dilute virus suspension more clear cut results would be obtained, in this test I decided to use a 1 : 100 dilution of virus. The complete brain of a mouse dying on the fifth day after an intracerebral injection of passage virus was suspended in 4·5 c.c. of saline. The suspension was centrifuged for thirty minutes at low speed. One-half of a cubic centimetre of the supernatant fluid was thoroughly mixed with 4·5 c.c. of saline. This concentration was called a one to one hundred dilution of the virus. Equal parts of sera and the dilute virus were thoroughly mixed and two drops of the mixture were injected immediately into

TABLE I.

Fate of mice injected intracerebrally with a mixture consisting of equal parts of 1 : 20 Virus and Immune Yellow Fever Sera.

History of serum				No. of mice injected	Number of mice which died. Time in days after injection.																No. of mice which lived	Remarks
No. of serum	Country of origin	Date of attack	Date of bleeding		1st day	2nd day	3rd day	4th day	5th day	6th day	7th day	8th day	9th day	10th day	11th day	12th day	13th day	14th day	15th day	16th day		
177	Brazil ...	June 13, 1928	Nov. 15, 1928	6	...	...	...	...	...	...	...	...	2	2	1	...	...	...	...	...	1	
152	Brazil ...	Aug. 2, 1928	Dec. 5, 1928	6	...	...	...	1	...	1	1	1	...	...	...	...	...	...	...	...	2	Serum re-tested, Experiment II.
179	Brazil ...	May 29, 1928	Dec. 5, 1928	6	...	2	1	...	...	...	...	...	...	...	...	...	...	...	1	...	2	
220	Brazil ...	Dec. —, 1928	Jan. 4, 1929	6	...	...	...	...	...	...	...	1	...	...	...	...	...	...	1		4	
85	Brazil ...	June 11, 1928	July 11, 1928	6	...	...	...	...	...	...	...	...	...	...	...	1	...	...	...	...	5	
388	Colombia ...	July 1, 1929	Aug. —, 1929	6	1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	5	
442	Laboratory infection, N.Y.	April 20, 1929	Jan. 7, 1930	6	...	...	...	...	...	...	2	...	...	1	...	...	...	...	...	...	3	Serum re-tested, Experiment III.
440	Laboratory infection, Bahia	Aug. 7, 1929	Dec. 28, 1929	6	...	1	...	...	...	...	1	...	...	...	...	...	...	...	...	1	3	
418	Laboratory infection, Bahia	Aug. 27, 1929	Nov. 29, 1929	7	...	...	...	...	...	...	1	1	...	...	...	...	...	1	...	...	4	
261	Africa ...	July 21, 1926	Jan. 5, 1929	6	...	...	1	...	...	2	1	1	...	...	...	1	...	...	...	...	0	Serum re-tested, Experiment III.
F	Normal ...	...	...	8	...	...	...	...	...	4	1	2	1	...	...	...	...	...	...	...	0	

each of six or eight mice. In all, five different sera were tested in this experiment. Three of these were from Colombia ; one (No. 152) had been tested in Experiment I and one was the serum from a normal individual. The results are shown in Table II.

The virus, though diluted, proved to be very virulent as shown by the death from the fifth to the seventh day of all the mice which received the mixture of normal serum and virus. Again, all the sera tested showed some protection, as manifested by the survival of some animals after all the controls had died. Serum 152, which in Experiment I showed protection in only two mice, in this experiment definitely showed protection in all six. Serum 390 showed very slight protection and was tested again in Experiment III.

The protective action of serum 394 was considered to be quite marked although no mice injected with a mixture of this serum and virus were alive at the end of three weeks. The two mice which died on the second and third days respectively can be excluded as being of no value. One mouse died on the seventh day and the remaining three died on the fourteenth, sixteenth, and eighteenth days. The average time of death of the controls was 6·1 days after injection, whereas the average time of death of the mice which received the mixture of serum 394 and virus was 13·7 days. In these calculations, the mice which died on the second and third days are excluded.

EXPERIMENT III

In this experiment the neutralizing action of four sera was tested. Serum 442 had been tested in Experiment I and was considered to be quite potent. Sera 261 and 390 had been tested in Experiments I and II respectively and showed very doubtful protection. Serum R 193 was the serum of a rhesus monkey which had been injected with two mouse brains of the thirty-first passage which had been stored in the cold room at –8° C. for 161 days. The rhesus showed no rise in temperature for thirty days following the injection of virus and remained well until he was accidentally killed four months after injection during the process of bleeding him from the heart.

All four sera were tested against a 1 : 100 dilution of virus and

TABLE II.

Fate of mice injected intracerebrally with a mixture consisting of equal parts of 1 : 100 Virus and Immune Yellow Fever Sera.

History of serum				No. of mice injected	Number of mice which died. Time in days after injection.																	No. of mice which lived	Remarks
No. of serum	Country of origin	Date of attack	Date of bleeding		1st day	2nd day	3rd day	4th day	5th day	6th day	7th day	8th day	9th day	10th day	11th day	12th day	13th day	14th day	15th day	16th day	17th day	18th day		
390	Colombia ...	June 7, 1929	Aug. —, 1929	6	...	...	...	...	...	2	2	...	2	...	...	...	...	...	...	...	...	...	0	Serum re-tested, Experiment III.
391	Colombia ...	July 20, 1929	Aug. —, 1929	6	...	...	...	...	...	2	1	1	...	...	...	...	...	1	...	...	...	...	1	
394	Colombia ...	April —, 1929	Aug. —, 1929	6	...	1	1	...	...	...	1	...	...	...	...	...	...	1	...	1	...	1	0	
152	Brazil ...	Aug. 2 1928	Aug. 22, 1928	6	...	...	...	...	...	...	...	...	...	1	...	1	...	...	...	...	...	...	4	
H	Normal ...	...	...	8	...	1	...	...	1	4	2	...	...	...	...	...	...	...	...	...	...	...	0	

two (Nos. 442 and R 193) were tested against a 1 : 10 dilution of virus as well.

The interpretation of the results is rather difficult on account of the fact that one of the control mice which had received a mixture of normal serum and 1 : 100 dilution of virus died on the twelfth day after a very prolonged period of illness. This mouse became ill on the seventh day and lingered on in a paralytic condition until death. This is a very unusual occurrence. It seems probable that this mouse received by accident an injection of a very minute amount of virus, as all the other mice injected with the same material were dead on the eighth day. With the exception of serum 390, all the sera tested against the 1 : 100 dilution protected one or more mice for at least twenty-one days. All the mice injected with the mixture of serum 390 and virus died, but on an average later than the controls. Excluding mice which obviously died too early to be of any significance the average time of death of the mice which received a mixture of serum 390 and virus is 9·8 days after injection, whereas the average time for the controls is 7·9 days.

The results obtained with serum 442 in this experiment are noteworthy. All six mice injected with a mixture of this serum and a 1 : 10 dilution of virus died during approximately the same range of time as the controls. When tested against a 1 : 100 dilution three mice died and three mice lived. This serum had been tested before in Experiment I in which a 1 : 20 dilution of virus had been used with results similar to those obtained with a 1 : 100 dilution in this experiment. The difference in results is in all probability due to the difference in virus content of different mouse brains or to the inadequacy of the technique employed in the preparation of a uniform virus suspension.

The serum of Rhesus 193 proved to be unusually potent in neutralizing power. All six mice injected with a 1 : 100 dilution of virus and this serum were alive at the end of three weeks when the experiment was terminated. The development of such a potent serum in this monkey suggests that the animal had been solidly immunized. It is to be regretted that this rhesus was accidentally killed during the process of bleeding as it would have been of great interest to test his immunity against virulent monkey virus.

TABLE III

Fate of mice injected intracerebrially with mixtures consisting of equal parts of 1 : 10 and 1 : 100 virus and sera.

Serum	Dilution of virus	No. of mice injected	Number of mice which died. Time in days after injection.														No. of mice which survived
			1st day	2nd day	3rd day	4th day	5th day	6th day	7th day	8th day	9th day	10th day	11th day	12th day	13th day	14th day	
442	1 : 10	6	...	...	...	...	...	1	3	1	...	...	...	1	...	...	0
R193	1 : 10	6	...	...	...	1	...	...	...	2	1	...	...	...	...	...	2
442	1 : 100	6	...	...	1	...	1	1	...	...	...	...	...	...	...	...	3
R193	1 : 100	6	...	...	...	...	...	...	...	...	...	...	...	...	...	...	6
390	1 : 100	6	...	1	...	...	1	...	...	...	1	...	1	2	...	...	0
261	1 : 100	6	...	1	1	...	...	...	1	...	...	1	1	...	...	1	1
Normal	1 : 100	8	...	...	...	1	...	...	5	1	...	...	...	1	...	...	0
Saline	1 : 100	6	...	...	...	...	...	1	4	...	1	...	...	...	...	...	0

EXPERIMENT IV

Summarizing these protection tests it may be seen that all the immune yellow fever sera showed a certain amount of protection against the mouse virus. It must be emphasized that the protection test as done is a very severe one.

Having established the fact that sera from yellow fever immunes will protect mice against the yellow fever virus adapted to mice, I decided to determine if possible the optimum conditions in order to get more clear cut results. With this in mind, serum from a laboratory infection as well as a normal serum were tested against a preparation of virus under different conditions. The results are summarized in Table IV. Three procedures were followed. (1) A 1 : 10 dilution of virus was mixed with an equal quantity of serum and the mixture placed in the incubator at 37·5° C. for one half-hour. (2) Serum and a 1 : 10 dilution of virus were mixed in equal amounts and the mixture injected immediately into mice. (3) Serum and a 1 : 100 dilution of virus were mixed in equal quantities. The mixture was allowed to stand at room temperature for thirty minutes. In all three procedures six mice were injected with each mixture. The results show plainly that under optimum conditions a very clear cut result is obtained. Thus all the mice which received the mixture of equal parts of immune serum and a 1 : 100 dilution of virus lived, whereas all the control mice died on the seventh and eighth days after injection.

SUMMARY

Thirteen convalescent human yellow fever sera were tested for their neutralizing action on the yellow fever virus adapted to mice. All showed a greater or less extent of protection.

REFERENCE

THEILER, MAX (1930). Studies on the Action of Yellow Fever Virus in Mice. *Ann. Trop. Med. and Parasitol.*, **24**, 249-272.

TABLE IV.

Fate of mice injected intracerebrally with mixtures consisting of equal parts of normal and immune sera, and 1 : 10 and 1 : 100 virus.

Procedure	Serum	No. of mice injected	Number of mice which died. Time in days after injection.													No. of mice which survived
			1st day	2nd day	3rd day	4th day	5th day	6th day	7th day	8th day	9th day	10th day	11th day	12th day	13th day	
Equal parts of serum and 1 : 10 virus mixed. 37° C. for half hour	Immune	6	...	...	...	...	...	...	...	...	1	...	...	...	1	4
	Normal	6	...	...	...	...	2	3	1	...	...	...	...	...	...	0
Equal parts of serum and 1 : 10 virus mixed. Injected immediately.	Immune	6	...	...	...	...	...	...	...	...	1	1	...	...	...	4
	Normal	6	...	...	...	...	...	1	2	3	...	...	...	...	...	0
Equal parts of serum and 1 : 100 virus mixed. Room temperature half hour.	Immune	6	...	...	...	...	...	...	...	...	...	...	...	...	...	6
	Normal	6	...	...	...	...	...	...	4	2	...	...	...	...	...	0

EXPERIMENTAL FELINE TRYPANOSOMIASIS WITH ESPECIAL REFERENCE TO THE EFFECT OF SPLENECTOMY

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It has been shown that in domestic cats preliminary splenectomy exercises a most profound influence in aggravating the course of experimental Piroplasmosis (Davis, 1929). It was therefore considered of interest to observe the effect of removal of the spleen on a subsequent trypanosome infection in the cat.

The extent to which the spleen may participate in the defence mechanism of the reticulo-endothelial system as a whole may be expected to vary in different animals according to the relative size of the organ in the species in question. According to Cannon and McClelland (1929) the ratio of the spleen-weight to body-weight is highest in the white rat (0·26 per cent.) and in man and dog (0·25 per cent.). The spleen of the cat, according to Krumbhaar, quoted by these authors, is only 0·05 per cent. by weight of the total body-weight. In fifteen healthy Khartoum cats, however, that were splenectomized consecutively, the average ratio of spleen-weight to body-weight was 0·189 per cent.

The cat, moreover, was deemed a suitable animal for the investigation of the part played by the spleen in Trypanosomiasis on account of the fatal albeit sub-acute nature of the infection produced in this animal by trypanosomes of the *brucei* type. Kanthack, Durham, and Blandford (quoted by Laveran and Mesnil (1907)) give the incubation period as five days and state that death occurs in twenty-two to twenty-six days after an infection during which the number of trypanosomes present in the blood show marked daily fluctuations.

The results of splenectomy in experimental Trypanosomiasis recorded by various investigators are conflicting. Bradford and Plimmer (1902) concluded that rabbits, dogs, cats, and rats from which the spleens had previously been removed died more quickly from *T. brucei* infections than did normal controls. In the case of the cat, however, only one splenectomized animal was observed : it died in twelve days while the average period of survival for control animals was twenty days. Laveran and Mesnil (1902), on the other hand, claimed that splenectomy of a white rat had no influence on a subsequent *T. brucei* infection. Sauerbeck (1905) and Rodet and Vallet (1906 and 1907) supported Bradford and Plimmer's claims, but Laveran and Thiroux (1907) working with *T. evansi* infections were unable to detect any departure from the normal course of infection in the case of two rats and five guinea-pigs that had been previously splenectomized. Massaglia (1907) confirmed these findings. Regendanz and Kikuth (1927) found that splenectomy of white rats rendered subsequent *T. lewisi* infections severer than usual. They concluded that the reaction product of Taliaferro is chiefly produced by the spleen. Knowles and Das Gupta (1928) found that splenectomy performed on three rabbits already infected with *T. evansi* was without influence on the course of the disease. Kligler (1929) studied the effect of splenectomy on white rats infected with *T. evansi*. He found that the operation activated an intense Bartonella anaemia and therefore treated the rats with Salvarsan. He reported a complete absence of resistance to infection in splenectomized rats when the trypanosomes were inoculated into the peritoneum, but when the inoculum was administered subcutaneously the effect of splenectomy was insignificant and the infection pursued its normal sub-acute course. Linton (1929) reported that splenectomy had no effect on the duration of life of guinea-pigs infected with *T. equiperdum*. Perla and Marmorston-Gottesman (1930) state that if splenectomized white rats are infected with *T. lewisi* forty-eight days after the operation, i.e., after recovery from the *Bartonella* infection, the trypanosome infection is heavier than in normal rats, and that both the reproduction inhibiting product and the lytic factor are depressed.

In the present experiments as strains of *T. brucei* and *T. rhodesiense* were available they were both used. The patho-

genicity of these trypanosomes for small animals is said to be identical (Wenyon 1926), but as no previous studies appear to have been recorded concerning the course of *T. rhodesiense* infection in the cat, observations on this head were considered to be not without interest.

The strain of *T. brucei* was originally isolated from a cow in the Nuba Mountains Province of the Sudan in 1927. The *T. rhodesiense* strain was isolated in 1926 from a case of sleeping sickness in the Southern Sudan. The original discovery of *T. rhodesiense* in the Sudan was recorded by Archibald (1922). Both strains have since been maintained in white rats and gerbils.

Throughout the experiments daily trypanosome counts were made at the same hour whenever the organisms were sufficiently numerous, the blood being obtained from ear veins. The technique recommended by Kolmer (1915) was employed, the blood being diluted with the following mixture :—

Formalin	...	...	2 c.c.
Glacial Acetic Ac.	...	...	2 c.c.
Carbol Fuchsin	...	...	2 c.c.
Distilled water	...	...	94 c.c.

It was found advisable to make up the fluid fresh each day. The dilutions were made in ordinary haemocytometer pipettes, 'red' or 'white' ones being chosen according to the degree of intensity of the infection. The counts were made in a Neubauer counting chamber. In the appended tables the figures indicate the number of trypanosomes per cubic millimetre of blood. When trypanosomes were present but too scanty for counting a plus sign is used.

The cats were kept in separate cages and fed on milk and raw meat, conditions which an extensive experience of experimental cats has proved suitable for the maintenance of health.

In the first experiment four cats were inoculated with *T. brucei* and a similar number with *T. rhodesiense.* Two of the cats in each series had been splenectomized six days previously. The inoculum in each case was administered subcutaneously and consisted of 0·1 c.c. of a citrated suspension of gerbils' blood containing 15,000 trypanosomes.

Reference to Table I shows that, in the cats, both strains of trypanosome produce fluctuating sub-acute infections lasting from

eleven to thirty-three days. It will be seen that splenectomy appears to exercise no significant influence on the duration of the infections. Furthermore, the evident variation in the period of survival of the normal cats renders manifest the danger of drawing conclusions based on slight departures from an average survival period of experimental animals, except when the number of animals used

TABLE I.

Showing daily trypanosome counts in normal and splenectomized cats infected with *T. brucei* and with *T. rhodesiense*.

T. brucei series

Date	Cat No. 1 (850 gms.)	Cat No. 2 (1,270 gms.)	Cat No. 3 (1,950 gms.)	Cat No. 4 (750 gms.)
29.7.30	...	...	Splenectomy	
5.8.30	Inoculated subcutaneously 15,000 *T. brucei* each			
	Trypanosomes per cubic millimetre.		Trypanosomes per cubic millimetre.	
8.8.30	—	+	+	+
10.8.30	1,500	+	51,000	89,000
11.8.30	+	+	85,000	78,000
12.8.30	+	—	42,000	17,000
13.8.30	14,000	+	+	6,000
14.8.30	+	12,600	1,800	33,000
15.8.30	+	27,000	46,000	67,000
16.8.30	600	Dead	140,000	Dead
17.8.30	+	...	28,000	...
18.8.30	18 000	...	1,200	...
19.8.30	64,000	...	1,800	...
20.8.30	74,000	...	4,600	...
21.8.30	18,600	...	—	...
22.8.30	28,400	...	+	...
23.8.30	34,000	...	+	...
24.8.30	68,000	...	+	...
25.8.30	1,400	...	400	...
26.8.30	600	...	1,600	...
27.8.30	—	...	1,200	...
28.8.30	—	...	19,000	...
29.8.30	—	...	43,000	...
30.8.30	1,200	...	138,000	...
31.8.30	16,200	...	216,000	...
1.9.30	33,000	...	240,000	...
2.9.30	1,700	...	350,000	...
3.9.30	1,200	...	Dead	...
4.9.30	—	...	...	...
5.9.30	4,800	...	...	...
6.9.30	29,000	...	...	...
7.9.30	85,000	...	...	...
8.9.30	Dead	...	...	...

TABLE I—*continued.*

T. rhodesiense series

Date	Cat No. 5 (1,040 gms.)	Cat No. 6 (720 gms.)	Cat No. 7 (1,250 gms.)	Cat No. 8 (800 gms.)
29.7.30	—	—	Splenectomy	
5.8.30	Inoculated subcutaneously 15,000 *T. rhodesiense* each			
	Trypanosomes per cubic millimetre.		Trypanosomes per cubic millimetre.	
8.8.30	+	+	—	+
10.8.30	600	800	+	+
11.8.30	26,000	36,000	1,200	2,600
12.8.30	34,000	48,000	28,000	42,000
13.8.30	400	40,000	45,000	62,000
14.8.30	3,200	52,000	32,000	20,000
15.8.30	55,000	50,000	6,600	15,200
16.8.30	66,000	72,000	+	1,800
17.8.30	15,000	32,000	4,600	7,000
18.8.30	Dead	1,800	10,200	14,200
19.8.30	...	20,000	22,000	20,000
20.8.30	...	16,600	80,000	48,000
21.8.30	...	+	56,000	72,000
22.8.30	...	0	Dead	150,000
23.8.30	...	+	...	240,000
24.8.30	...	+	...	78,000
25.8.30	...	Dead	...	Dead
26.8.30	...	...	...	...
27.8.30	...	...	...	...
28.8.30	...	...	...	...
29.8.30	...	...	...	...
30.8.30	...	...	...	...
31.8.30	...	...	...	...
1.9.30	...	...	...	...
2.9.30	...	...	...	...
3.9.30	...	...	...	...
4.9.30	...	...	...	...
5.9.30	...	...	...	...
6.9.30	...	...	...	...
7.9.30	...	...	...	...
8.9.30	...	...	...	...

TABLE II.

Showing daily trypanosome counts in normal and splenectomized cats inoculated with *T. rhodesiense* subcutaneously compared with those in a similar series inoculated intra-peritoneally.

Date	Cat No. 9 (870 gms.)		Cat No. 10 (850 gms.) splenectomy 1.8.30		Cat No. 11 (1,740 gms.) splenectomy 27.7.30
15.9.30	Inoculated subcutaneously 6,000 *T. rhodesiense* each				
	Tryps. per c.mm.	Mean length and standard deviation in microns	Tryps. per c.mm.	Mean length and standard deviation in microns	Tryps. per c.mm.
17.9.30	−	...	−	...	0
18.9.30	−	...	−	...	0
19.9.30	+	...	−	...	0
20.9.30	+	...	+	...	+
21.9.30	6,500	25·25±2·73	2,800	24·7±2·79	20,200
22.9.30	2,800	24·8 ±2·83	10,600	24·1±2·34	96,000
23.9.30	+	...	3,200	24·6±2·66	34,000
24.9.30	−	...	1,100	...	−
25.9.30	−	...	4,600	25·2±3·12	−
26.9.30	−	...	800	...	−
27.9.30	800	24·7 ±3·24	1,000	...	−
28.9.30	2,100	25·24±2·63	+	...	600
29.9.30	+	...	−	...	3,000
30.9.30	+	...	−	...	7,000
1.10.30	+	...	+	...	19,000
2.10.30	+	...	+	−	40,000
3.10.30	600	...	+	...	24,000
4.10.30	2,400	...	−	...	3,000
5.10.30	3,400	24·6 ±3·55	+	...	600
6.10.30	2,000	23·5 ±3·34	+	...	−
7.10.30	1,100	...	+	...	+
8.10.30	2,600	20·6 ±2·96	+	...	+
9.10.30	+	...	+	...	+
10.10.30	−	...	600	...	400
11.10.30	−	...	2,000	24·9 ±3·26	+
12.10.30	+	...	1,700	24·6 ±2·88	+
13.10.30	+	...	5,600	24·1 ±3·43	600
14.10.30	2,500	25·3 ±3·1	17,200	22·5 ±3·5	+
15.10.30	1,200	25·5 ±2·98	1,900	23·8 ±2·99	2,200
16.10.30	1,300	...	+	...	14,000
17.10.30	6,400	25·9 ±2·79	+	...	54,000
18.10.30	13,400	25·5 ±2·72	+	...	94,000
19.10.30	18,000	24·8 ±2·65	+	...	102,000
20.10.30	4,800	23·5 ±2·6	+	...	75,000
21.10.30	+	...	10,200	26·1 ±2·78	66,000
22.10.30	−	...	62,000	25·5 ±2·89	114,000
23.10.30	+	...	174,000	26·2 ±2·35	118,000
24.10.30	4,200	...	236,000	25·2 ±3·32	126,000
25.10.30	34,000	24·0 ±2·89	228,000	23·3 ±3·28	239,000
26.10.30	15,000	24·7 ±2·87	27,000	24·7 ±3·57	142,000
27.10.30	+	...	4,300	24·3 ±3·43	Dead
28.10.30	3,200	23·3 ±2·84	75,000	24·9 ±2·38	...
29.10.30	19,000	22·9 ±3·07	Dead	...	...
30.10.30	24,000	24·8 ±2·98	...	...	...
31.10.30	6,800	24·5 ±2·96	...	...	...
1.11.30	−	...	...	...	...
2.11.30	+	...	...	...	...
3.11.30	3,700	25·1 ±2·91	...	...	...
4.11.30	108,000	24·8 ±3·17	...	...	...
5.11.30	66,000	25·2 ±3·32	...	...	...
6.11.30	97,000	24·7 ±3·33	...	...	...
7.11.30	38,000	25·2 ±3·03	...	...	...
8.11.30	Dead	...	...	...	...
9.11.30	...	...	...	...	...
10.11.30	...	...	...	...	...
11.11.30	...	...	...	...	...
12.11.30	...	...	...	...	...
13.11.30	...	...	...	...	...
14.11.30	...	...	...	...	...
15.11.30	...	...	...	...	...
16.11.30	...	...	...	...	...

TABLE II—*continued.*

Date	Cat No. 12 (950 gms.)	Cat No. 13 (1,650 gms.)	Cat No. 14 (1,900 gms.)	Cat No. 15 (1,350 gms.)
			Splenectomy. 2.8.30	Splenectomy. 4.8.30
15.9.30	Inoculated intra-peritoneally 6,000 *T. rhodesiense* each.			
	Tryps. per c.mm.	Tryps. per c.mm.	Tryps. per c.mm.	Tryps. per c.mm.
17.9.30	−	−	−	−
18.9.30	−	+	−	−
19.9.30	+	−	+	11,400
20.9.30	2,200	−	+	+
21.9.30	10,100	−	+	+
22.9.30	1,500	−	+	−
23.9.30	−	−	−	+
24.9.30	−	−	+	+
25.9.30	−	+	+	16,000
26.9.30	+	1,100	3,800	21,000
27.9.30	−	+	−	6,400
28.9.30	−	+	−	1,100
29.9.30	+	+	+	+
30.9.30	+	−	−	+
1.10.30	−	−	−	−
2.10.30	+	−	+	−
3.10.30	600	−	+	+
4.10.30	+	−	+	+
5.10.30	−	−	+	1,600
6.10.30	+	+	+	6,200
7.10.30	+	+	+	22,000
8.10.30	2,800	−	+	36,000
9.10.30	400	+	2,800	16,000
10.10.30	5,200	+	13,000	3,600
11.10.30	21,000	−	23,000	+
12.10.30	8,800	−	18,000	+
13.10.30	16,000	−	24,000	+
14.10.30	8,000	−	22,000	3,400
15.10.30	5,000	−	22,000	5,200
16.10.30	9,000	−	46,000	10,800
17.10.30	22,000	−	48,000	17,400
18.10.30	8,000	−	38,000	24,000
19.10.30	11,000	−	40,000	35,000
20.10.30	5,000	−	44,000	54,000
21.10.30	26,000	+	69,000	78,000
22.10.30	46,000	−	87,000	112,000
23.10.30	56,000	−	222,000	62,000
24.10.30	20,000	+	356,000	Dead
25.10.30	35,000	−	190,000	...
26.10.30	5,200	−	Dead	...
27.10.30	Dead	−	...	...
28.10.30	−	−	...	...
29.10.30	...	−	...	...
30.10.30	...	+	...	...
31.10.30	...	+	...	...
1.11.30	...	3,800	...	...
2.11.30	...	5,200	...	...
3.11.30	...	+	...	...
4.11.30	...	+	...	...
5.11.30	...	−	...	...
6.11.30	...	+	...	...
7.11.30	...	11,800	...	...
8.11.30	...	13,000	...	...
9.11.30	...	21,000	...	...
10.11.30	...	36,000	...	...
11.11.30	...	79,000	...	...
12.11.30	...	136,000	...	...
13.11.30	...	212,000	...	...
14.11.30	...	52,000	...	...
15.11.30	...	90,000	...	...
16.11.30	...	176,000 Dead	...	...

is large enough to permit of adequate statistical treatment. The variation in the body-weight of the cats all of which received an equal infecting dose appears to be without significant effect on the course of the infection. The trypanosome counts do, however, display a tendency to be definitely higher during the final stages of infection in the case of the de-splenated cats than in that of the normal controls.

In view of Kligler's findings, to which reference has already been made, it was decided to repeat the experiment and observe the effect of the intra-peritoneal route of inoculation. In this second experiment *T. rhodesiense* only were employed. Three cats, two of which had been splenectomized, were inoculated subcutaneously, and four cats, two of which had been splenectomized, were inoculated by the intra-peritoneal route. In all cases the inoculum consisted of a citrated suspension of gerbils' blood each dose containing 6,000 trypanosomes. In addition, in the case of two of the cats, Taliaferro's (1922, 1923) statistical method of analysing the nature of resistance exerted by the host was applied. This method involved the measurement by means of a micro-projection apparatus of from 80 to 100 trypanosomes on each day that they were sufficiently numerous in the blood. The measurements were made by the usual method of drawing free-hand through each trypanosome a centre-line which was subsequently measured by means of dividers. From these data the mean length and standard deviation were worked out for each sample, using the usual formula $\sigma = \sqrt{\frac{\Sigma d^2}{n}}$. From this the coefficient of variation is readily obtained from the formula $\frac{100\ \sigma}{M}$. The films for measuring the trypanosomes were at first fixed in osmic acid vapour and methyl alcohol, subsequently being stained with Giemsa. Latterly, however, thin films were air-dried and stained with Leishman. The dry hot atmosphere of Khartoum causes very rapid drying and comparisons showed that measurements made from films prepared by either method did not differ materially.

Taliaferro considers the coefficient of variation in the lengths of a sample of trypanosome an index of their reproductive activity, so that if in spite of a moderately high reading, the numbers of

trypanosomes in the blood remain constant the resistance of the host is probably due to trypanocidal bodies. This appears to be the case in all sub-acute trypanosome infections so far investigated by this method with the exception of *T. lewisi* infections in the white rat in which a significant drop in the coefficient of variation coincides with the decrease in the number of parasites. This is interpreted as evidence of a reproductive inhibiting product.

In the case of cats 9 and 10 reference to Figures 1 and 2 shows that the coefficient of variation fluctuates between 10 and 16 per cent., thus indicating a state of continued reproductive activity of a moderate degree. The resistance displayed by both cats appears then to rest chiefly with trypanocidal anti-bodies.

The course of the infections in the cats of this second experiment is more prolonged and in the early stages more benign than in those of the first experiment. Whether this is due to the infecting dose being smaller it is difficult to say. The strains were the same as those used earlier and had in the meantime been passaged in gerbils. The cats Nos. 9, 10, 11, that were inoculated subcutaneously, behaved comparably to those of the first series. The splenectomized cats survived nearly as long as the control cat, but the final rise in the number of trypanosomes in the blood of these two cats is more than twice as high as in that of the control. In the four cats that were inoculated by the intra-peritoneal route, there is again no decisive evidence that splenectomy decreases the period of survival but there is, however, the suggestive relatively heavy increase in the number of parasites just before death.

Throughout the observations posterior nuclear forms of the parasite were never seen.

The progress of the disease in the cats was characterised by progressive emaciation, irregular pyrexia, and keratitis.

SUMMARY AND CONCLUSIONS

1. Experimental *T. brucei* and *T. rhodesiense* infections have been observed in a small number of cats, some of which had been previously splenectomized.

2. Both strains of trypanosome produced sub-acute infections fluctuating in intensity and of variable duration.

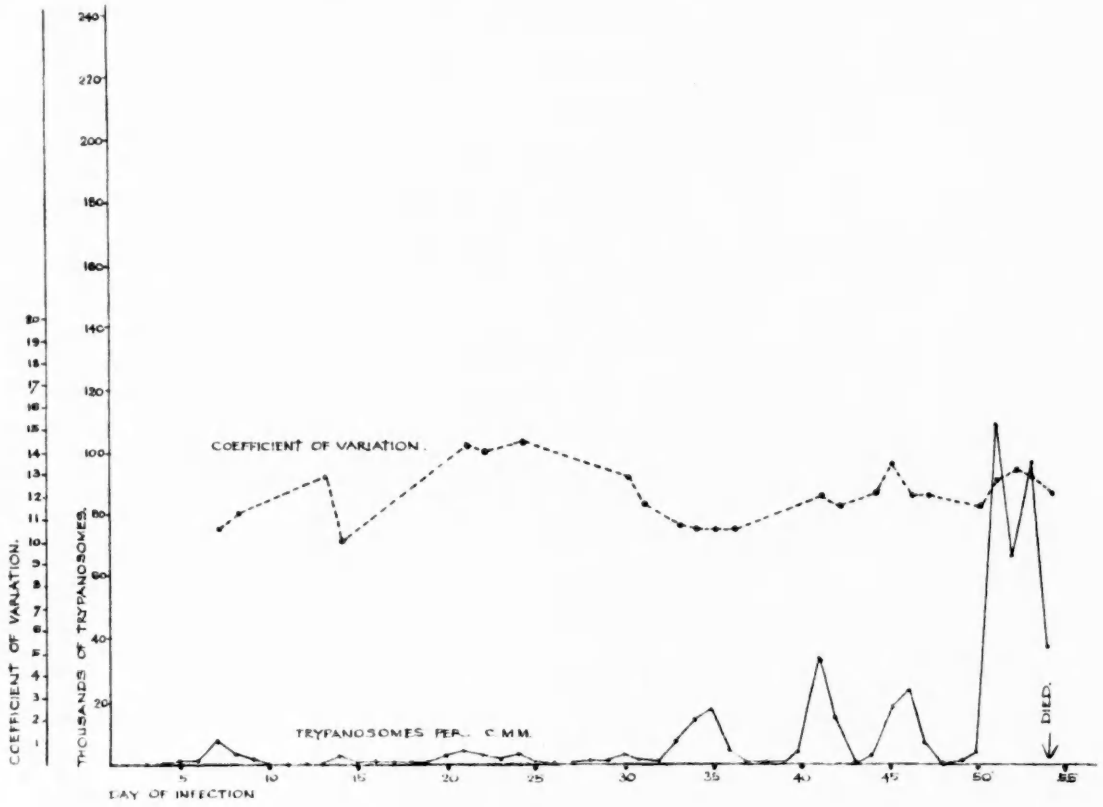

FIG. 1. The course of infection with *T. rhodesiense* in a normal cat (No. 9).

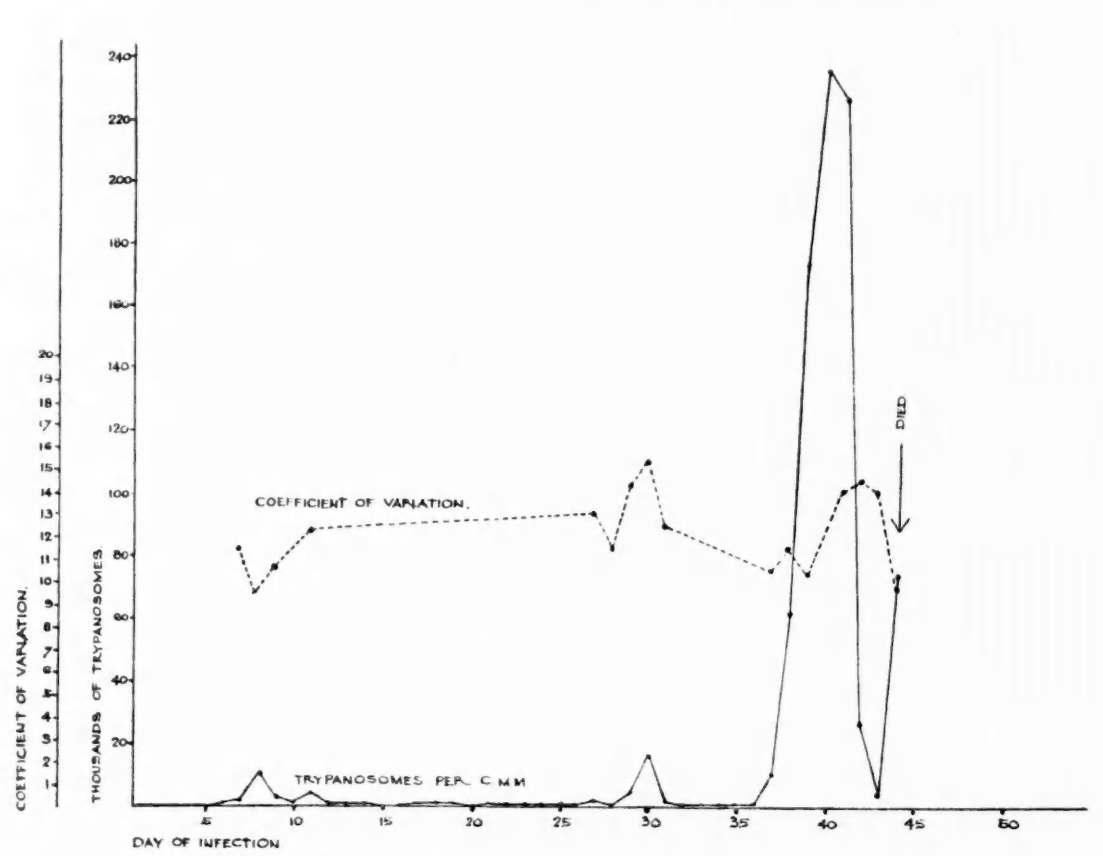

FIG. 2. The course of infection with *T. rhodesiense* in a splenectomized cat (No. 10).

3. The average survival period of the splenectomized cats did not differ significantly from that of the normal controls. The number of trypanosomes in the peripheral blood shortly before death appeared to be significantly higher in the splenectomized cats.

4. *T. rhodesiense* infections in normal and in spleenless cats inoculated by the intra-peritoneal route were compared with those in a similar series inoculated subcutaneously. No significant difference was noted in the duration or intensity of the infections in the two series.

5. Taliaferro's statistical method of analysing the nature of the resistance of the host was applied to the study of *T. rhodesiense* infections in a normal and in a splenectomized cat. In both cases the coefficient of variation indicated a state of continued reproductive activity of moderate degree.

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NOTES ON THE SERUM CALCIUM OF INHABITANTS OF TRINIDAD, B.W.I.

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From an analysis of the blood serum of eighteen Indians and eight Europeans, Hughes, Shrivastava and Sahai (1929) found the calcium content of the serum higher in normal healthy individuals living in the Punjab than in the inhabitants of temperate climates where the normal average is ca. 10·28 mg. per 100 c.c. They explained the cause of these high values (in Indians 12·51 and in Europeans 11·74) as due to excessive solar ultra-violet radiation, producing vitamin D from the ergosterol present in the skin and this in turn leading to an increased absorption of calcium from the intestinal contents. Among Indians there is usually more of the skin exposed and the effect upon them is usually more marked. On the other hand Henderson in Kenya (1926-27) had found the average serum calcium of 90 natives (prisoners, shamba boys) without obvious disease to be 9·4 mg./100 c.c. and stated ' the general figure for natives is somewhat low '. Kelly and Henderson (1930) in a subsequent work on 42 apparently healthy prisoners in Nairobi Prison, Kenya Colony, obtained a yet lower average, 9·2 mgm.—' a considerable number being definitely subnormal figures,' e.g., 7·3 mg. In their method of calcium determination they accepted the suggestion of Pincussen and Schimmelffung and allowed precipitation to take place over-night. It should be noted, however, that in a very detailed investigation of dietary supplement in relation to the calcium requirements of five growing East African native boys they stated that ' the serum calcium values were initially normal and remained so throughout the experiment.'

In view of the discrepancy in the figures obtained in Africa and India the results of the blood calcium estimation of seventy-four (74) individuals in Trinidad may be of some interest. The method of Kramer and Tisdall was employed, but precipitation was allowed to take place in twenty minutes. All reagents were contained in Pyrex glassware and B. D. H. chemicals used. Each specimen was either done twice—once by each of us and the average taken—or if done only once the end point of titration was agreed upon by both of us. The results are tabulated in disease groups (pp. 93-95).

In Trinidad the inhabitants supplying material for the above analysis were drawn mainly from the artisan class of the City of Port-of-Spain. Their bodies are not exposed to the sun to the extent generally obtaining among the class referred to by Hughes, Shrivastara and Sahai in India and they conform in the main to the European standard of dress in the tropics. Their diet, largely through economic reason, is deficient in minerals and accessory factors. Among these individuals the average serum calcium of all cases (74) is 10·74 mg. per 100 c.c. This figure can be regarded as normal even for the inhabitants of temperate climates. It is certainly not low despite the fact that precipitation of the calcium was allowed to take place in twenty minutes and not in 12-24 hours. If the objection to the former time raised by Pincussen and Schimmelffung be correct the results might have been higher than those obtained in temperate climates and have approximated more closely the figures for India. Kelly and Henderson (1926) in their work in Nairobi on the effect of supplemental diets on the human subject, concluded that calcium is one of the most important constituents in a supplementary dietary mixture and further that a rise in the calcium content of the blood followed an increase of calcium in a supplemental diet, though they add that when an individual is living on a low calcium diet the amount of calcium in the blood may not necessarily reflect this deficiency. In Trinidad, though the diet of these subjects is deficient in calcium contents, the average serum calcium is not low. This result may be due to either the counteracting effect of ultra violet solar radiation or, as suggested by Kelly and Henderson, to imperfect calcium blood response. Examination of the figures according to disease groups is of some interest.

TABLE I.

Healthy individuals.

Sex.	Age.	Disease.	Quantity.	Race*
			Mgms.	
M.	19	Nil ... ... ?	11·7	N.
F.	40	„	12·0	E.I.
M.	30	„	10·7	N.
M.	30	„	12·0	N.
M.	32	„	12·2	N.
M.	34	„	10·4	N.
M.	29	„	10·0	N.
M.	35	„	9·9	N.
M.	30	„	10·6	N.
M.	38	„	11·9	N.
M.	21	„	10·7	N.
M.	30	„	10·7	N.
M.	30	„	9·5	E.I.
F.	33	„	10·3	E.I.
M.	45	„	10·8	N.
M.	31	„	10·6	N.
M.	39	„	10·3	E.I.
F.	28	„	10·7	E.I.
F.	50	„	10·0	N.
F.	27	„	10·7	M.
F.	60	„	10·7	M.
M.	23	„	10·5	E.
M.	26	„	10·7	E.
F.	35	„	10·3	M.

Total = 24 Cases.
Average = 10·7.
Max. = 12·2.
Min. = 9·5.

* N. = Negro. E. = European. E.I. = East Indian. M. = Mixed.

TABLE II.

Sex	Age	Disease	Quantity	Race*
			Mgms.	
M.	14	Chronic ulcer foot	11·7	N.
F.	23	Chronic ulcer leg	11·1	M.
M.	79	Chronic ulcer leg	11·2	M.
M.	59	Chronic ulcer leg	11·3	M.
M.	17	Chronic ulcer foot	11·9	N.
M.	55	Chronic ulcer foot	12·0	M.
F.	15	Chronic ulcer arm	10·3	E.I.
M.	43	Chronic ulcer foot	10·7	N.
F.	23	Chronic ulcer foot, general debility	9·3	M.
F.	30	Chronic ulcer leg, general debility	9·2	N.
F.	23	Sloughing ulcer leg, general debility	8·9	N.
F.	52	Chronic ulcer leg, general debility	8·4	N.
F.	26	Chronic ulcer foot, general debility	8·9	N.
F.	20	Chronic ulcer legs, general debility	8·9	N.
F.	30	Chronic ulcer leg, general debility	9·1	M.
M.	34	Chronic ulcer leg, general debility	10·3	N.

Average all ulcer cases = 10·20. Max. = 12·0. Min. = 8·4.
Average ulcer cases with general debility = 9·12.
Maximum ulcer cases with general debility = 10·3.
Minimum ulcer cases with general debility = 8·4.

TABLE III.

Nephritis.

Sex	Age	Disease	Quantity	Race*
			Mgms.	
M.	25	Acute parenchymatous (hydraemic)	7·7	M.
M.	27	Subacute parenchymatous (hydraemic)	6·8	M.
M.	22	Chronic parenchymatous (hydraemic)	9·0	N.
M.	22	Chronic parenchymatous (hydraemic)	8·0	N.
F.	34	Chronic parenchymatous (hydraemic)	8·4	N.
F.	27	Chronic parenchymatous (hydraemic)	8·5	N.
F.	18	Pregnancy (acute hydraemic)	9·1	N.
F.	20	Pregnancy (acute hydraemic)	7·4	E.I.
F.	40	Chronic azotaemic	8·7	N.

Average = 8·18. Maximum = 9·1. Minimum = 6·8.

* N. = Negro. E. = European. E.I. = East Indian. M. = Mixed.

TABLE IV.

Dental Cases.

Sex	Age	Disease	Quantity	Race*
			Mgms.	
F.	23	Dental caries	13·5	N.
M.	19	Dental caries	10·0	M.
F.	21	Dental caries	9·8	N.
F.	23	Dental caries	7·7	N.
F.	32	Dental caries	10·4	M.
F.	29	Dental caries	10·1	N.

Average = 10·25. Maximum = 13·5. Minimum = 7·7.

TABLE V.

Other conditions.

Sex	Age	Disease	Quantity	Race*
			Mgms.	
M.	30	Chronic asthma	12·0	M.
M.	22	Chronic malaria	11·7	M.
F.	30	Acute arthritis (?)	11·6	M.
F.	24	Chronic valvular disease	10·6	N.
M.	22	Syphilis	10·6	E.I.
M.	30	Chronic bronchitis	13·2	N.
M.	32	Chronic periostitis	12·1	E.I.
F.	21	Syphilis	12·0	N.
M.	74	Senility (?)	10·0	M.
M.	38	Chronic diarrhoea	9·7	M.
F.	31	Acute nephritis 8 years ago	10·7	N.
M.	20	Eczema toes	10·7	M.
F.	38	Bronchial asthma	9·4	M.
M.	30	Exfoliative dermatitis	9·5	M.
M.	43	Urticaria	9·5	M.
F.	30	Post. eclampsia	8·7	E.I.
F.	20	Nursing mother	11·9	E.I.
F.	17	Nursing mother	10·7	N.
F.	25	Ankylostomiasis	10·7	N.

Average = 10·8. Maximum = 13·2. Minimum = 8·7.

Average of 74 cases = 10·15 mg./100.

* N. = Negro. E. = European. E.I. = East Indian. M. = Mixed.

Normal Healthy Subjects. Among 24 apparently healthy individuals engaged in their ordinary occupation the average serum calcium is 10·7 mg./100 c.c., the lowest figure being 9·5. Davies and De Wesselow give the lowest average figure for temperate climates as 9. In no instance in this series was this low level reached and in only three instances was the highest average of 11 for temperate climates exceeded. Hughes and his co-workers in India explained the high calcium level among their normal cases to excessive solar radiation acting upon an exposed skin surface. Henderson and Kelly, on the other hand, working among native Africans presumably with much of their bodies also exposed obtained low results. In Trinidad, among a people living on a deficient diet 10° north of the Equator, our figures approximated the level of those for temperate climates. It would seem therefore—as is further borne out by the experimental work of Harvard and Hoyle (1928) and Harris and Stewart (1929)—that there are unknown factors responsible for the concentration of calcium in the blood.

Ulcer Cases. Henderson (1927) in Kenya, found the average figure of 32 cases before treatment to be 9·9, a higher figure than the average of 9·6 for all cases and for normal natives. These formed a group among 140 cases of tropical ulcers in which Vincent's fusiform bacillus and a spirochaete were present. An analysis of possible etiological causes based on detailed and carefully controlled dietary experiments led him to the conclusion that ' the incidence of tropical ulcers appears to be closely associated with the condition of malnutrition which is general in the tribes, and more particularly among the males, in those tribes where it occurs and to vary with seasonal fluctuations in food supply.' Variations in the diet and supplementary additions led to an increase in the body weight of the subjects which was followed by an improvement in the condition of the ulcers. Henderson was impressed by the degree of malnutrition from which some of the cases suffered, pointing to not only a qualitative food deficiency but also a quantitative one.

In Trinidad the ulcer cases may be divided into two groups. One comprises individuals who, apart from the presence of ulcers, appeared well. These ulcers, as well as those of the second group, did not present the clinical picture of sloughing tropical ulcers. Among these cases the serum calcium was never below 10. In most

cases it was above 11, i.e. above the average normal. These results, therefore, agree with those of Henderson in Kenya who, however, states that ' both calcium and phosphorus are higher in ulcer cases than in normal natives and since the higher blood calcium in ulcer cases is not associated with any improvement in condition over normal cases it is probably associated with the very high blood phosphate.' The Trinidad results among this group of ulcer cases considered in contrast with the second group is, however, of interest. In this latter group the patients were obviously feeble and poorly nourished. From inquiry into their diets and from eighteen years' experience of the dietary possibilities of the class of hospital patients one can assert that their diets are deficient in quality and quantity. No attempt was made to exclude the possibility of parasitic infestation or syphilis as a cause of the ' general debility ' which was apparent on the most casual observation. In every instance but one the serum calcium was definitely below the average for the normal and for all cases in Trinidad.

Nephritis. For the purpose of this investigation a patient with the clinical signs, and showing albumen and tube casts, was regarded as suffering from nephritis. About 60 per cent. of the calcium of the blood is in a diffusible form and about 40 is non-diffusible. This non-diffusible calcium is in combination with the protein of the blood. In renal disease there is a reduction in the serum protein and consequently the combined calcium also suffers diminution. In temperate climates the serum calcium in hydraemic nephritis lies between 8-8 mg./100 c.c. In Trinidad it is seen from Table III that in all the cases of nephritis there was reduction of the serum calcium to below the normal average and in every case except one to below the normal average of 9 for nephritis cases in temperate climates. In one instance the figure fell as low as 6·8.

Carious Teeth. It is commonly accepted that the coloured races of mankind possess strong well-formed non-carious teeth. This belief is contrary to our experience of hospital patients in Trinidad. Malformed and carious teeth are the rule and not the exception among the poorer classes here. This fact, in conjunction with their defective diets, confirm the experimental work of Mellanby, who has shown that ' the dietetic substances which tend to promote well-developed and well-calcified teeth are generally those which

are known to have a high content of vitamin D and to a less extent of A.' The figures obtained here in subjects of carious teeth show considerable variations—from 13·5, the highest figure in the whole series of 74 cases, to 7·7, one of the lowest—and suggest the absence of a direct relationship between serum calcium and teeth formation.

SUMMARY

1. The serum calcium in Trinidad of 74 persons—healthy and sick—averaged 10·15 mg. per 100 c.c.

2. Of 24 normal healthy individuals it averaged 10·74.

3. There was a definite diminution in the serum calcium of persons with general debility and ulcers, whereas persons with ulcers and otherwise in good health show a rise above the normal.

4. Nephritis cases show a low serum calcium—in some cases very low.

5. In dental cases the serum calcium shows great variation.

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THE SECRETORY GLANDS OF THE CERCARIA OF *S. HAEMATOBIUM*

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When Leiper (1915-1918) investigated the life history of *S. haematobium* and published his discoveries and observations, he gave no detailed account of the anatomical features of the cercaria.

Faust (1919) described three pairs of mucin glands in the cercaria of *S. haematobium* in specimens from Natal. The glands began in the region of the acetabulum on either side and were said to empty their contents by mucin gland ducts at the margin of the oral sucker. These mucin glands had loosely scattered granules in their cytoplasm and large nuclei, the granules being acidophilic. No mucin glands other than the above mentioned three pairs were found. Later, Faust (1920) reaffirmed the number of mucin glands in *S. haematobium* as being only the three pairs described above. Manson-Bahr and Fairley (1920) stated that the cercaria of *S. haematobium* had three pairs of large clear cells with acidophil protoplasm and clear-cut nuclei. They called these cells ' poison cells ' or salivary glands.

Khalil (1922) commented that no reliable account of the anatomy of the cercaria of *S. haematobium* was then available. Bettencourt and Da Silva (1922)* described in the cercaria of *S. haematobium* three pairs of glands with large acidophil granules in the cytoplasm occupying nearly the whole of the posterior portion of the body.

Blacklock and Thompson (1924), in a paper published in May of that year, stated that the posterior two-thirds of the body of the cercaria were almost entirely occupied by pairs of unicellular glands, five on each side, each with a single and definite nucleus. The proto-

* Abstract only consulted *Trop. Dis. Bull.*, **20**, No. 3, 1923, p. 218.

plasm of the cells contained granules which were of large size in the anterior two pairs and of small size in the posterior three pairs of glands. The two anterior pairs of coarsely granular salivary glands were large and flask-shaped, lying just anterior to the ventral sucker ; the three posterior pairs of finely granular salivary glands lay behind the anterior two pairs and were smaller in size and overlapped each other. They found that the affinity of the glands for stains was variable and not constant and they suggested that this variability of staining capacity might be due to variations in the secretory activity of the glands.

Their cercaria were identified as being that of *S. haematobium* on the following grounds :—

(1) The incidence of *S. haematobium* in the human host, i.e., epidemiological data.

(2) The presence of naturally infected *Physopsis globosa* (Morelet).

(3) Experimental infection of these snails.

(4) The positive results of experimental infection in laboratory animals.

In a subsequent paper written in June (1924), these authors stated that they had now had the opportunity of seeing Khalil's paper (1922), in which he gave a description of the salivary glands of the cercaria of *S. mansoni*. They, commenting upon the similarity of Khalil's and their own description of the glands of *S. mansoni* and *S. haematobium* respectively, drew attention to the fact that the most recent studies disclosed no constant morphological character nor staining reaction by which the cercaria of *S. haematobium* and *S. mansoni* could be distinguished.

Bettencourt and Da Silva (1925) immediately issued a paper in a pamphlet form, specifically a criticism of the work of Blacklock and Thompson (1924), on the anatomy of the cercaria of *S. haematobium*. They stated that after seeing the papers of these authors, they had repeated their former work which was published in 1922 and that the repetition left them in no doubt that their own previous results were amply confirmed. They reiterated their conclusions and stated positively that in *S. haematobium* there were only three pairs of unicellular glands, all of one type, disposed symmetrically, with a protoplasm rich in acidophilic granulations, and each containing a

large basophilic nucleus and a nucleolus. From these glands, ducts of the same number ran forwards and terminated at the anterior sucker.

These results were based on the examination of the cercaria from naturally infected *Planorbis metidjensis*, the snail, which, they affirmed, was beyond dispute the intermediate host of *S. haematobuim* in Tavira. On infecting mice with such cercariae, adult males and females of *S. haematobium* were recovered. After declaring the confirmation of their work by that of Faust, Manson-Bahr and Fairley, and the unmistakable surety of their own cercaria being that of *S. haematobium*, they criticised the work of Blacklock and Thompson, casting doubt on the cercaria of these authors being that of *S. haematobium*.

Faust (1926) revised his former work on human schistosome cercariae, and after a careful study of material from South Africa and Palestine and the preparation of fresh sections, stained with Delafield's haematoxylin and counterstained with Best's alum carmine, he then declared : ' the writer is able to state that he is in full agreement with Blacklock and Thompson with respect to the number of glands in the cercaria of *S. haematobium*.' He also found that the glands were of two types : two pairs with large nuclei and granular acidophilic cytoplasm and three pairs with basophilic reaction.

In a footnote he stated that the differentiation in the secretory glands was constantly found in the cercaria from the germ ball stage to maturity. At the end of the review of this paper in the *Tropical Diseases Bulletin*, it is said : ' Faust had not seen the paper by Bettencourt and De [*sic*] Silva when the present paper was written.'

Faust (1930), in the first edition of his book on Human Parasitology, again described in the cercaria of *S. haematobium*, two pairs of anteriorly situated unicellular glands with granular contents and oxyphilic reaction and three pairs of posteriorly situated unicellular glands with homogeneous contents and a basophilic reaction.

Professor Blacklock handed over to me three bottles of livers of infected snails, *Physopsis globosa* (Morelet), which he collected in Sierra Leone, the cercaria in them being of the *S. haematobium* type prevalent in the Protectorate. The livers had been preserved in alcohol for nearly a year. I passed some of the livers from each

batch through absolute alcohol and xylol, embedded them in paraffin, cut sections, and stained them with haematoxylin and counter-stained with eosin. The cercaria in the first batch together with the parenchymatous tissue of the snail took the stain very badly. The parenchymatous cells were degenerate and the protoplasm was fragmented and granular, hardly taking up the stain ; the nuclei also took it badly.

Cercaria were seen cut in different directions and the cercarial tissue and salivary glands showed a good deal of degeneration. The cells of the salivary glands were vacuolated and some of them contained granular detritus, but the outline of the cells was quite apparent ; the nuclei and nucleoli were quite visible, although they took the stain badly. In the sections from these livers the salivary glands did not show definite granules, but were vacuolated and some were empty due to degenerative changes resulting possibly from delay in preservation. I went carefully over the sections, counting the number of glands in each. The maximum number of glands counted in any section of one cercaria was eight. I might mention that all the eight glands were not seen in one plane under the microscope ; focussing at different levels was necessary in order to see the outline of the glands and their nuclei superimposed on each other.

The sections made from the other two batches of livers took the stain satisfactorily and showed that the material was well preserved. They nearly all contained cercariae cut in different planes and directions, and of these several showed a variable number of glands, according to the level and direction of the section, through the body of the cercariae. On examination of the glands I was immediately struck by the presence of two definite and distinct types.

(1) Large cells situated at the level of the ventral sucker with their protoplasm coarsely granulated. The granules resemble those of the eosinophil cells of the blood and take the eosin stain intensely, i.e., they are oxyphilic. Each of these cells possessed a large nucleus and a distinct nucleolus.

(2) The other type of cell is much smaller than the above and is situated posterior to them. Each gland has a nucleus and a nucleolus, but the protoplasm is either homogeneous or very finely granular and takes the haematoxylin stain, i.e., it is basophilic.

A large number of the cercarial sections showed the two types of glands in each cercaria, the numbers of each varying according to the plane and direction of the sections. Some cross-sections showed only one type of gland due to the section being made at the level of either type of gland.

I went over my sections very carefully counting the numbers of each type of gland in each section. I was unable to find more than four large granular acidophilic glands or more than six finely granular or homogeneous, small basophilic glands in any one cercarial section. The definite conclusion reached was that there are five pairs of these secretory glands in the cercaria, two anteriorly placed pairs about the level of the ventral sucker, large in size and coarsely granular, having oxyphilic granules, and three posteriorly placed pairs of small size and finely granular or homogeneous and basophilic.

This conclusion regarding the number of glands in the cercaria is borne out by the figures given. My results are thus in agreement with those of Blacklock and Thompson and also with Faust's (1926) amendments.

Haematoxylin with eosin as a counterstain gave very good results so far as the granules were concerned ; there was no variability in the staining results by this method as the acidic and basic staining reactions of the two types of glands were constant. Although my results agree with Blacklock and Thompson in the number and appearance of the glands, my staining reactions differ from theirs in that mine were not variable. In this connexion one point must be taken into consideration, i.e., my results are based on the staining reactions of sections from immature cercariae in the snail's liver and the results of Blacklock and Thompson were based on mature cercariae, so that possibly this reaction may vary according to the secretory activity or developmental phases of the cercaria.

Khalil, staining the cercaria of *S. mansoni*, states that although he noticed the granulations in the fresh specimens, these disappear on staining and that this may be due to the staining reagents dissolving them.

It is of interest to observe that there is a great similarity in the description of the number and type of the glands of the cercaria of *S. mansoni* by Khalil and the description of the glands of *S. japonicum* by Takahashi (1928). The latter author states that the

anterior two pairs of glands differ morphologically and microchemically from the three posterior pairs. If this description be accepted, the conclusion drawn by Blacklock and Thompson in 1924, that there are no constant morphological characters by which to distinguish the cercariae of *S. haematobium* and *S. mansoni*, may now be extended to include *S. japonicum*.

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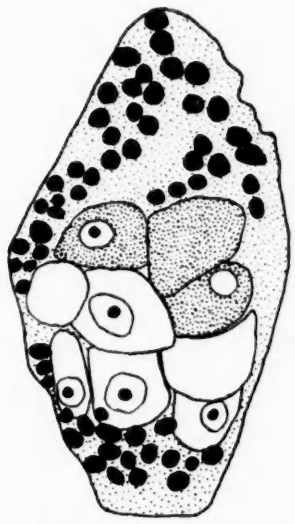

FIG. 1. Longitudinal section of a cercaria showing nine glands, three of the granular oxyphilic anterior type, two of which show the nuclei, and six of the non-granular basophilic posterior type, four of which show nuclei. Camera lucida × 630.

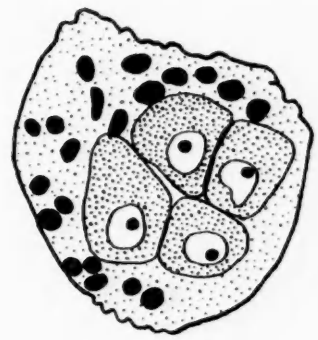

FIG. 2. Cross section of a cercaria at the level of the large granular oxyphilic anterior glands, four showing nuclei. Camera lucida × 630.

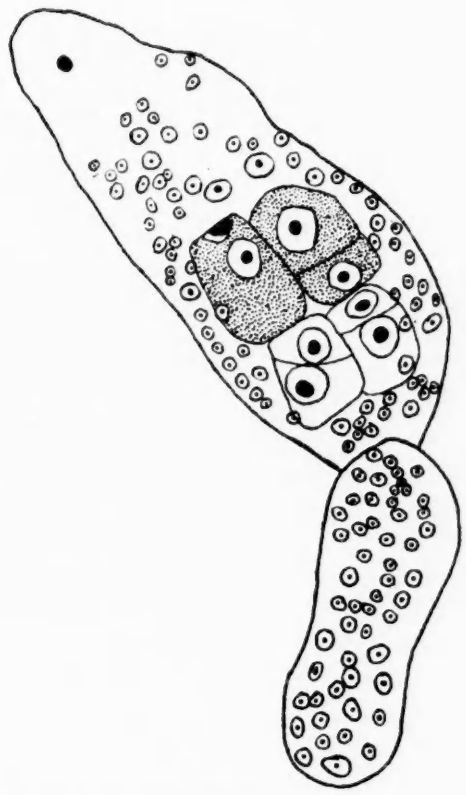

FIG. 3. Longitudinal section of a cercaria with tail. Showing seven glands, three of the large granular oxyphilic anterior type and four of the non-granular small basophilic posterior type, all showing nuclei. Camera lucida × 630.

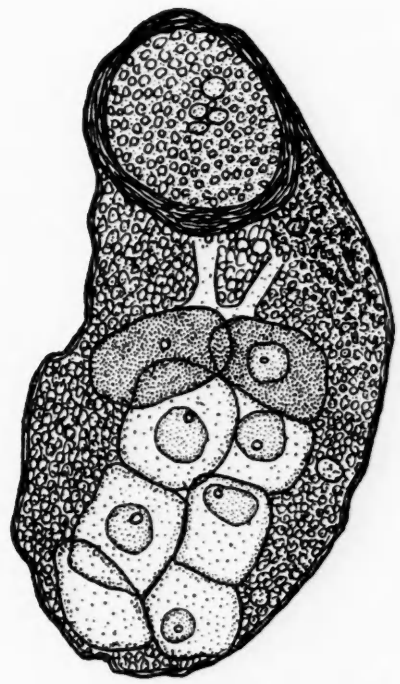

FIG. 4. Longitudinal section of a cercaria showing eight glands, two of the granular oxyphilic anterior type, one showing its nucleus, and six of the non-granular basophilic posterior type, five of which show the nuclei. Camera lucida × 630.

ON THE IDENTITY OF *TRICHOSTRONGYLUS AXEI* (COBBOLD, 1879) RAILLIET and HENRY, 1909, AND *T. EXTENUATUS* (RAILLIET, 1898) RANSOM, 1907

BY

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HISTORICAL

In 1879, Cobbold gave the name *Strongylus Axei* to a small worm, with a brief notice of its discovery by Mr. Axe in the mucous membrane of the stomach of a donkey (*Equus asinus*). The same worms were subsequently observed in three other donkeys brought to the dissecting room of the Royal Veterinary College, London. Later, in 1884, Cobbold published a short description of the parasite. In 1886 he published a more lengthy and detailed description—given below—accompanied by figures.

'*Strongylus Axei.* (Cobb.)

'*Body* filiform, narrowed in front and behind; *mouth* simple—with short oesophagus and strong chitin layer; *hood* [bursa] bilobed, with deeply cleft anterior [ventral] ray and widely separated divisions; trunk of the posterior [dorsal] ray united to its fellow, bifurcate at the end; spicules three, the two larger nearly equal, with a small third or accessory piece intercalated; *tail* of female ending in a postanal cone, finely pointed; *vulva* within the lower sixth of the body. Length of male nearly $\frac{1}{6}''$ (strictly $\frac{15}{100}$ in.) [3·75 mm.]; of female $\frac{1}{5}''$ ($\frac{20}{100}$ in.) [4 mm.]

'*Hab.* Mucous membrane of the stomach of the ass (*Equus asinus*). Whilst the extreme transparency of the worm readily permits the ova and other organs to be measured *in situ*, the tubal and ovarian filaments, as well as the corresponding elements in the male, entirely escape observation. The lumen of the oesophagus is clearly traceable, a dark line below it representing the closed pharynx; but I could find no trace of any bulb or other line of separation between the pharynx and the chylous intestine. I suspect the arrangement is the same as obtains in *Strongylus Douglassii*. The mid-gut is well marked, as is also the rectum at its anal end.

'The pattern of the hood is distinctive, well pronounced, and symmetrical. The widely separated divisions of the anterior ray [ventral rays] are thumb-and-finger-like, the upper digitoid [the ventro-ventral ray] being comparatively short and narrow, whilst the lower [the latero-ventral ray] is closely applied to the succeeding ray, except at the end which is turned upward. The antero-lateral or second [externo-lateral] ray is paramount and directed downward. The middle, third or lateral [medio-lateral + postero-lateral] ray proper is of moderate size and deeply cleft into equal halves. The postero-lateral or fourth [externo-dorsal] ray is narrow, straight and placed well apart. The posterior or fifth [dorsal] ray is narrower, and has the shaft united to its fellow of the opposite lobe throughout its upper two-thirds, the lower end bifurcating into subequal divisions. If the ray pattern as a whole be compared with that seen in the ostrich strongyle, the affinity of the two species becomes apparent. The general form and disposition of the rays are similar throughout, the most striking difference being that of the cleavage of the posterior [dorsal] ray, which in *Strongylus Douglassii* is three cleft. It is interesting to observe that whilst all the rays in the ostrich strongyle are relatively stouter than they are in *Strongylus Axei*, they nevertheless individually bear towards each other similar proportions in both species. Thus the thumb-and-finger-like form of the anterior [ventral], the paramount antero-lateral [externo-lateral], the moderate sized middle [medio-lateral + postero-lateral], the isolated postero-lateral [externo-dorsal] and the slender posterior rays [dorsal ray] of *S. Axei* have their counterpart, ray for ray, in *S. Douglassii*. Nevertheless the distinctions already noticed have full specific value apart from those affecting other organs. Quite recently another and larger species of Strongyloid worm (*Sclerostoma struthionis*) [*Cadiostomum struthionis* (Horst, 1885) Railliet and Henry, 1911] has been discovered by Dr. Horst in an ostrich (*Struthio molybdophanes*). In Horst's entozoon the ray-pattern is altogether unlike either of the above-named species.

'The eggs of *Strongylus Axei* are relatively large, and one can clearly observe the process of yolk-segmentation through the finely-striated integument of the body-wall. The large and conspicuous spicules are ploughshare-shaped, with a tendency towards division of the shaft, the intercalated small spicule being simple and slightly winged at the centre. This accessory piece can only be seen by strongly pressing the cover-glass, or by dissection. In the ostrich strongyle I did not find a third spicule; but I infer that it is present from the general correspondence of the larger organs in both species.

'The facts above stated will perhaps be further emphasized by the accompanying approximate measurements:—Head, $\frac{1}{1000}''$ to $\frac{1}{750}''$ [25μ to 33μ] broad; tail, above the spicules $\frac{1}{250}''$ [100μ], at the narrowest part above the anus of the female $\frac{1}{680}''$ [37μ]; base of the tail-cone $\frac{1}{1000}''$ [25μ] in breadth; length $\frac{1}{350}''$ [71μ]; hood $\frac{1}{200}''$ [125μ] in length by $\frac{1}{80}''$ [312μ] in breadth; large spicules $\frac{1}{240}''$ [104μ] and $\frac{1}{230}''$ [109μ] respectively, accessory piece $\frac{1}{450}''$ [55μ] in length; eggs $\frac{1}{250}''$ to $\frac{1}{225}''$ [100μ to 111μ] in length by an average of $\frac{1}{400}''$ [62μ] in breadth; distance from the anus to the vulva $\frac{1}{33}''$ [760μ].'

The contents of the square parentheses are the present writer's; in the case of the names of the rays the plan proposed by Looss (1905) and which is generally recognised by all recent authors is adopted. British measurements have been converted into the usual metric measurements. Cobbold's statement that the accessory piece in *T. Axei* cannot be seen unless the coverslip is strongly pressed or by dissection is not true because it is easily seen if the worm is properly

rolled ; his surmise that *T. Douglassii* has an accessory piece is now an established fact. The description and figures of *T. Axei* given by Cobbold are not accurate enough to establish a definite diagnosis for the species under consideration. The shape of the spicules and of the dorsal ray in the male are of the utmost importance in differentiating species of this genus, together, of course, with the arrangement and shape of the other rays. Cobbold's 1886 description of *T. Axei* and his figures of the spicules and rays (with the exception of the dorsal ray) could be applied to any species of the genus and is thus quite inadequate for the purpose of classification to-day.

Yet so far as I am aware this description is the only one which exists of *T. Axei ;* Travassos (1921) greatly abbreviates Cobbold's description, copies some of his figures and adds that the parasite has been only twice reported, once by Cobbold and once by Mazzanti. Gertrud Theiler (1923) copies the descriptions given by Travassos. Both these authors make a mistake in the date of the naming of the parasite (*T. Axei*) making it 1880 instead of 1879. It is true that Cobbold described and figured the parasite in 1886 ; but in 1879 he named it and gave an indication which satisfied articles 21 and 22 of the International Code of Zoological Nomenclature. Travassos also considers *T. Axei* and *T. extenuatus* as distinct species.

Railliet and Henry (1909), referring to the classification of the genus *Trichostrongylus,* write as follows :—

' *Trichostrongylus* Looss, 1905—Espèce type : *Tr. retortaeformis* (Zeder) ; autres espèces : *Tr. pergracilis* (Cobbold) ; *Tr. instabilis* (Raill.) ; *Tr. probolurus* (Raill.) ; *Tr. vitrinus* Looss ; *Tr. capricola* Ransom ; *Tr. tenuis* (Mehlis) ; *Tr. Axei* (Cobbold) [*Str. tenuissimus* Mazzanti, *Str. gracilis* McFadyean, ? *Str. Placei* Place, *Str. extenuatus* Raill.].'

It is not clear from the above whether Railliet and Henry consider the species contained in the square parentheses as synonyms of *T. Axei,* or whether they are casting doubt on their identity with *T. Axei.* It is worth while to state in passing that *Str. Placei* Place, 1893, is now regarded as identical with *Haemonchus contortus* (Rud., 1803) Cobb., 1898, and not with *T. Axei.* The only description which was furnished by Place is that the parasites were ' Strongyle-like worms 1-3 cms. in length ' which cause outbreaks of chronic diarrhœa in young cattle. Now *T. Axei* or any recognised member of the genus *Trichostrongylus* never exceeds one centimetre in length.

Pil'ers (1915) writes :

'*Trichostrongylus axei* (Cobbold), (*Strongylus gracilis* McFad., *S. extenuatus* Railliet).—This species was discovered by Cobbold in 1886, and re-described by McFadyean in 1896 . . . . The male is from 3·5 to 4·5 mm. in length . . . . The female is 4·5 to 5 mm. long, and the vulva is about 1 millimetre from the tip of the tail. It is a comparatively common and injurious species and is often associated with other members of its own or other genera.'

He apparently copies MacFadyean and Railliet and Henry and makes a mistake in the date of naming the parasite. On a previous page he writes about the round worms of the alimentary canal of the horse :

'*Trichostrongylus axei* (Cobbold).—It is not infrequently found in the stomach, where it gives rise to a gastritis very similar to that of ruminants ;'

and about the parasitic gastritis in the horse :

'The horse is probably more often affected with this condition than is supposed. *Trichostrongylus axei* (*T. extenuatus*, *Strongylus gracilis*) is usually the cause of the disease. The stomach often appears to be smaller than normal, but its walls are greatly thickened. The mucous membrane is usually corrugated and covered with an abnormal amount of mucus, which contains great numbers of the parasites. The first few inches of the duodenum may be similarly affected. Adenomatous-like formations on the gastric lining have been stated by some authors to be caused by this worm.

'*Symptoms.*—There is gradually increasing emaciation, anaemia, and a capricious appetite. The unthriftiness increases, and the appetite is almost lost for several days altogether. There may be slight colic-like pains. Cases usually progress until after three to six months they become cachetic and die.'

Pillers also writes the following about parasitic gastro-enteritis in cattle :—

'The genera responsible for this disease have already been mentioned. In this country it is, however, more particularly associated with *Haemonchus contortus*, *Ostertagia circumcincta*, *O. ostertagia* and *Trichostrongylus axei*. The other groups are at times present, with clinical pictures suggesting injurious effects ; thus, *Cooperia oncophora* has been shown to be numerous in some outbreaks of gastro-enteritis in calves. In cattle young animals are most likely to be attacked, and although the same usually applies to sheep, at times older animals are seriously infested, and especially is this so in pregnant ewes.

'*Symptoms.*—These are somewhat similar in all ruminants, but the following remarks apply more especially to sheep : If lambs are running with the flock, they will often be noticed to be thriving badly, on account of the lack of the dams' milk. There is nearly always diarrhœa, together with loss of flesh and appetite. The diarrhœa is often profuse, watery, and usually without any other visible discharges, such as mucus. With the rapid loss of flesh, the fleece along the back is elevated, harsh, and may finally become loose. The absence of appetite carries with it cessation of rumination. Anæmia, which is marked, is shown by pallor of the visible mucous membranes. Œdema of the intermaxillary space is often noticeable. After a few days, the patient spends much time on the ground, and all the symptoms become aggravated. In sheep death often takes place in from three days to three

weeks; but in calves the disease is not so acute. Bronchial worms very often coexist, and there are then corresponding thoracic symptoms. Even in the pure gastro-enteritis there is, however, a few degrees of fever in the early stages.

'*Lesions.*—The carcass shows all the indications of anæmia and emaciation. The contents of the abomasum are usually very liquid, and some portions of the mucous membrane are highly coloured. Similar changes are seen in the duodenum. The most important feature is the discovery of the parasites, either in the fluid contents, in the mucus, upon the mucous membrane, or embedded in the membrane itself. This can usually be accomplished by the dilution of a few drops of the material in a petri-dish, and examining in the dish or on a slide under the $\frac{2}{3}$-inch lens. It should be remarked that a similar dispute has arisen here as in the case of grouse disease as to the actual cause of the disease. Although a few authorities blame micro-organisms, which have gained access to the tissues on account of the worm infestation, the majority seem convinced that the worms are the most important factor.

'*Diagnosis.*—This must depend upon a number of animals being affected with symptoms as described above, and by the demonstration of the parasites. An animal is nearly always available for post-mortem examination when the veterinarian is consulted. The fourth stomach should have both orifices legatured before removal, and the viscus should, if possible, be examined when warm. In the absence of such an examination, the disease is likely to be confused with a number of other conditions, such as scour from non-verminous causes, tuberculosis, Johne's disease, and for the effects of some of the mineral or vegetable poisons.'

Ransom (1911) describes *T. extenuatus* among the nematodes parasitic in the alimentary tract of cattle, sheep and other ruminants. The same author again (1912) mentions *T. Axei* and *T. extenuatus* in a list of the members of the genus.

Leiper (1911) mentions *T. Axei* among the helminths parasitic in equines. The same author again (1913) writes in a check list of helminths parasitic in cattle: ? *T. axei*, *T. extenuatus* and ? *T. retortæformis*.

Boulinger (1915) mentions *T. extenuatus* in a list of nematode parasites observed in the alimentary canal of sheep in England. Cameron (1922) also mentions *T. extenuatus* in sheep in Britain.

Monnig (1925) describes a new species, *T. rugatus* and compares it with *T. instabilis* and *T. extenuatus* which are present in sheep in S. Africa. Later (1927), in a paper about the life-histories of *T. instabilis* and *T. rugatus* he mentions *T. extenuatus* as the third *Trichostrongylus* met with in sheep in S. Africa.

Yorke and Maplestone (1926) again regard the two species *T. Axei* and *T. extenuatus* as distinct species.

Baylis (1929) writes about *T. extenuatus*:

'Right spicule 0·085 to 0·095 mm. long, left spicule 0·11 to 0·12 mm., each giving off a slender, pointed, posteriorly directed process on its inner aspect near

the middle. Accessory piece 0·05 to 0·06 mm. long. Male 3·4 to 4·4 mm., female 4·6 to 5·5 mm. long. Hosts: ox, sheep, goat;'

and writes of *T. Axei*:

'Occurs on the mucosa of the stomach of the horse and donkey, where, according to Le Roux, it apparently sometimes causes superficial "ringworm-like" lesions. The male is 3·8 to 6 mm. in length, the female 5 to 8 mm. The spicules are 0·11 to 0·15 mm. long. The eggs measure 0·1 to 0·112 × 0·063 mm. This species bears a very close resemblance to *T. extenuatus*, of which possibly it is really a variety.'

From the above it will be clear that all these authors consider that *T. Axei* and *T. extenuatus* are distinct species.

In 1894, Penberthy wrote an article on 'Parasitic Gastro-enteritis, Diarrhœa and Anaemia of Cattle,' in which he dealt with the history, symptoms, etiology, morbid anatomy and treatment of these conditions. He attributes great importance to the presence in the fourth stomach of minute strongyles as the cause of this condition of persistent diarrhœa and wasting. From the description of the parasite published by Penberthy it is evident that this parasite must have been *T. Axei* (= T. *extenuatus*). In 1896 MacFadyean wrote a paper entitled 'Verminous Gastro-enteritis in Cattle,' in which he describes and photographs two worms, the first one (species I as he called it) is an *Ostertagia* and the second (species II) he named *Strongylus gracilis*. This name was found by Railliet (1898) to be pre-occupied by another *Strongylus gracilis* Leuckart, 1842, now *Heligmosomum gracile* (Leuckart, 1842) Railliet and Henry, 1909; and therefore Railliet re-named the parasite *Strongylus extenuatus*.

Before proceeding to give my evidence for the statement that *T. extenuatus* is identical with *T. Axei*, it may be well to enumerate briefly the pathological effect produced by this important parasite of domestic stock, as observed by MacFadyean: The constant symptoms were diarrhœa, emaciation and anæmia. In some cases the disease set in rather suddenly with violent diarrhœa and rapid loss of condition, while in others a period of unthriftiness preceded the onset of more acute symptoms. In most of the outbreaks only young animals (under two years old) fell victims, but cows of all ages may sometimes be attacked. The appetite was but little interfered with, and not rarely it was good up to the very last. The pulse, respiration and temperature were not markedly abnormal. Diarrhœa could be temporarily or permanently held in check by

the administration of astringent medicines, but this is not sufficient to effect a cure in animals already markedly wasted, the emaciation continuing although the diarrhœa had been arrested, and in spite of a liberal diet. Death sometimes resulted in a few weeks after the onset of decided symptoms, but in the case of animals housed and carefully attended to they sometimes survived for six months or more.

The post-mortem examination of animals killed at an advanced stage of this disease affords but little variety in the seat or nature of the lesions. The normal adipose tissue of the body is conspicuous by its absence, and the blood is thin and watery. Sometimes there is more or less œdema of the connective tissue, and a moderate amount of dropsical fluid in the serous cavities. As a rule, however, the emaciation was not accompanied by any notable degree of dropsy, either of the inter-maxillary space, dewlap or serous cavities.

In the majority of cases the mucous membrane has a pale anæmic appearance, and not infrequently this pallor is associated with marked œdema of the membrane.

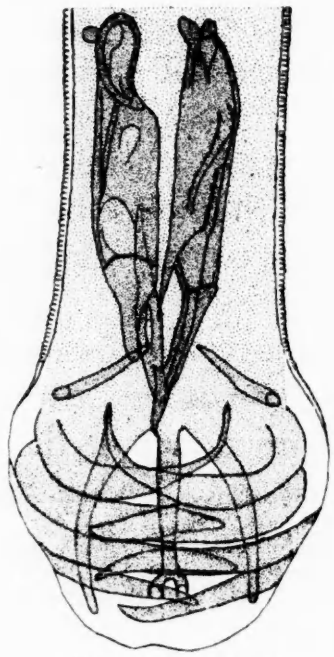
In some cases, particularly those of the more rapid type in young animals, the mucous membrane of the abomasum shows more or less inflammatory congestion. The mucous membrane of the small intestine in some cases shows scattered patches of congestion, or even petichial haemorrhages, but as a rule the small bowel is normal. The large bowel, particularly the caecum, is not rarely distinctly inflamed, and its mucous membrane may show numerous small haemorrhages.

THE MORPHOLOGY OF *T. AXEI* AND *T. EXTENUATUS*

Since Cobbold's description in 1886, any member of the genus *Trichostrongylus* found in a horse has been invariably called *T. Axei*, although, as I have shown, the description of *T. Axei* is not adequate. It has to be accepted that the *Trichostrongylus* found in the horse by all observers and called *T. Axei* by them and that the *Trichostrongylus* present in my material from the horse is, in fact, *T. Axei*.

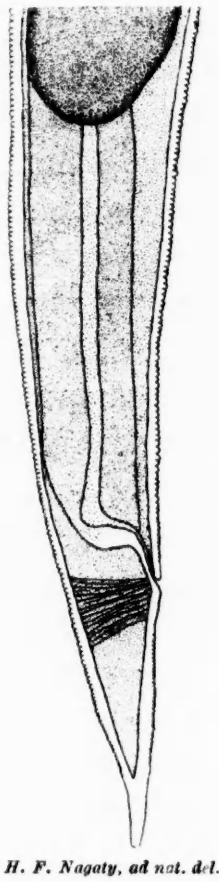
H. F. Nagaty, ad nat. del.

FIG. 1. Lateral view (right side) of the bursa of *T. Axei* from a horse. The dorsal ray is not shown. The posterior tips of the spicules and gubernaculum which should have appeared in this figure are omitted.



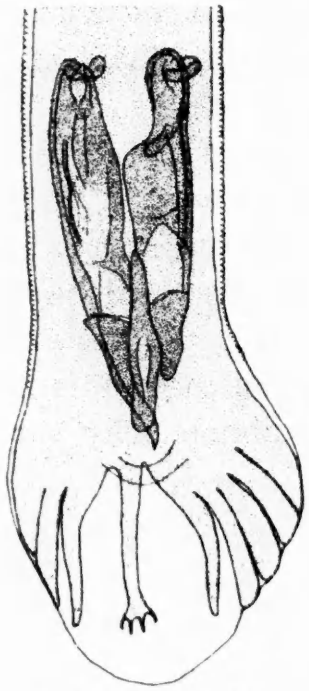
H. F. Nagaty, ad nat. del.

FIG. 3. Ventral view of the bursa of *T. Axei* from a horse, including the spicules and gubernaculum. The lateral lobes of the bursa are folded.



H. F. Nagaty, ad nat. del.

FIG. 5. The posterior end of a female *T. Axei* from a horse. Note the gradual narrowing of the body from the loop of the ovary to the tip of the tail.

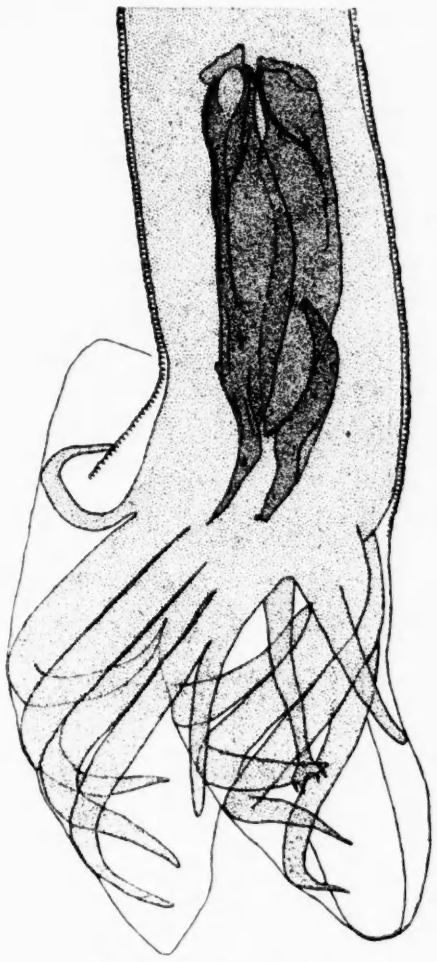


H. F. Nagaty, ad nat. del.

FIG. 2. Dorsal view of the bursa of *T. Axei* from a horse, including the spicules and gubernaculum. Note the characteristic shape of the dorsal ray, also the spicules and gubernaculum. It is noticed here that the bursa is slightly rolled towards the left side, so that the gubernaculum comes to lie between the two spicules. If the bursa is rolled to the right so that the dorsal ray is exactly in the middle line, the gubernaculum will appear dorsal to the right spicule.

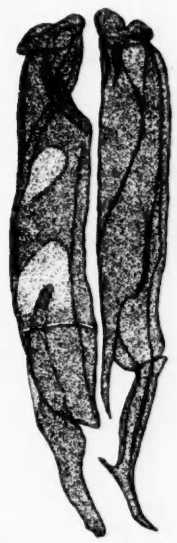
H. F. Nagaty, ad nat. del.

FIG. 4. Dorsal view of the spicules and gubernaculum of *T. Axei* from a horse. They are slightly rolled towards the left side.



H. F. Nagaty, ad. nat. del.

FIG. 6. Postero-lateral view of the bursa of *T. Axei* (=*T. extenuatus*) from a goat.

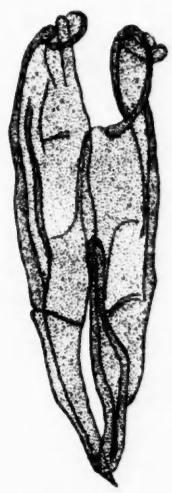


H. F. Nagaty, ad nat. del.

FIG. 7. Ventral view of the spicules and gubernaculum of *T. Axei* (=*T. extenuatus*) from a goat. Compare these with those in fig. 3 and note that in both figures the gubernaculum is dorsal to the right spicule.

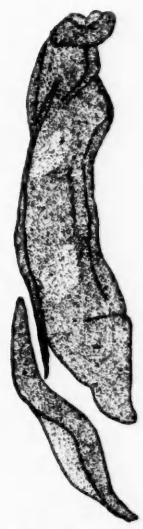
H. F. Nagaty, ad nat. del.

FIG. 8. Ventral view of the spicules and gubernaculum of *T. Axei* (=*T. extenuatus*) from a sheep, slightly rolled to the right.



H. F. Nagaty, ad nat. del.

FIG. 9. Dorsal view of the spicules and gubernaculum of *T. Axei* (=*T. extenuatus*) from a sheep; they are slightly rolled towards the left.



H. F. Nagaty, ad nat. del.

FIG. 10. Lateral view (right side) of the gubernaculum and right spicule of *T. Axei* (=*T. extenuatus*) from a goat.

H. F. Nagaty, ad nat. del.

FIG. 11. The posterior end of a female *T. Axei* (=*T. extenuatus*) from a sheep.

Figures 1, 2, 3, 5, 6 and 11 are magnified 422 times approx.
Figures 4, 7, 8, 9 and 10 are magnified 475 times approx.

Table giving measurements of the different organs in *Trichostrongylus Axei* (= *T. extenuatus*).

	Sex	Length in mm.	Diameter of the head in μ	Distance of excretory pore from head in μ	Length of oeso-phagus in μ	Distance of vulva from tip of tail in μ	Length of the slit of vulva in μ	Diameter of body at region of vulva in μ	Combined lengths of ovejectors in μ	Posterior ovary bends forwards at . . . from the tip of tail in μ
1	♂	3·211	8·8	132	?	...	...	...	...	...
2	♂	3·306	13·2	140·8	616	...	...	...	...	...
3	♀	3·629	8·8	132	594	703	52·8	44	277·2	154
4	♀	3·628	8·8	110	?	722	57·2	52·8	242	158·4
5	♂	2·717	13·2	149·6	554·4	...	...	...	...	...
6	♂	2·584	13·2	132	528	...	...	...	...	...
7	♂	3·097	13·2	136·4	?	...	...	...	...	...
8	♀	3·496	13·2	154	624·8	686·4	52·8	52·8	250·8	136·4
9	♀	3·268	13·2	140·8	668·8	646	44	57·2	242	189·2
10	♀	3·363	13·2	149·6	567·6	682	44	52·8	202·4	105·6
11	♀	3·306	13·2	136·4	514·8	646	44	61·6	233·2	118·8
12	♂	4·75	13·2	176	668·8	...	...	...	...	...
13	♂	3·648	13·2	132	665	...	...	...	...	...
14	♂	3·268	13·2	132	836	...	...	...	...	...
15	♂	3·515	13·2	140·8	?	...	...	...	...	...
16	♂	3·078	13·2	162·8	708·4	...	...	...	...	...
17	♂	3·325	13·2	149·6	567·6	...	...	...	...	...
18	♀	3·8	13·2	154	673·2	703	44	57·2	220	176
19	♀	4·009	13·2	158·4	734·8	722	52·8	57·2	250·8	202·4
20	♂	3·401	13·2	?	?	...	...	...	...	...
21	♀	3·99	13·2	145·2	779	779	52·8	52·8	264	176
22	♂	3·059	13·2	154	624·8	...	...	...	...	...
23	♂	3·42	13·2	158·4	704	...	...	...	...	...
24	♂	3·078	13·2	154	550	...	...	...	...	...
25	♂	2·945	13·2	149·6	638	...	...	...	...	...
26	♂	3·002	13·2	154	616	...	...	...	...	...
27	♀	3·99	13·2	154	690·8	792	57·2	52·8	281·6	171·6
28	♀	3·8	13·2	154	726	722	52·8	52·8	272·8	211·2
29	♀	3·933	13·2	154	?	703	48·4	57·2	272·8	158·4
30	♀	4·218	13·2	162·8	871·2	779	52·8	57·2	255·2	167·2
31	♀	3·705	13·2	140·8	?	703	52·8	52·8	290·4	215·6

Nos. 1-4 are from the stomach of sheep, England.
Nos. 5-11 are from a horse, Surrey.
No. 12 from the small intestine of goat, Bromsgrove, England.
Nos. 13-21 from the stomach of horse from the Federated Malay States.
Nos. 22-31 from the fourth stomach of an ox.

Table giving measurements of the different organs in *Trichostrongylus Axei* (= *T. extenuatus*).

	Sex	Anterior ovary bends backwards at . . . from head in mm.	Number of ova in anterior and posterior uteri	Length of tail in μ	Diameter of body at region of anus in μ	Diameter of body anterior to bursa in μ	Length of left spicule in μ	Length of right spicule in μ	Length of gubernaculum in μ	Length of dorsal ray
1	♂	...	...	...	...	48·4	110	88	57·2	52·8
2	♂	...	...	...	...	44	110	88	57·2	52·8
3	♀	1·443	3 and 2	70·4	22	...	...	...	...	...
4	♀	?	4 and 4	66	22	...	...	...	...	...
5	♂	...	...	...	...	57·2	110	88	52·8	44
6	♂	...	...	...	...	52·8	96·8	79·2	52·8	44
7	♂	...	...	...	...	48·4	110	88	57·2	...
8	♀	1·069	3 and 2	66	26·4	...	...	...	...	...
9	♀	998·8μ	4 and 3	70·4	26·4	...	...	...	...	...
10	♀	1·016	4 and 3	70·4	26·4	...	...	...	...	...
11	♀	1·153	2 and 3	61·6	26·4	...	...	...	...	...
12	♂	...	...	...	...	66	123·2	96·8	61·6	...
13	♂	...	...	...	...	57·2	114·4	92·4	61·6	...
14	♂	...	...	...	...	57·2	118·8	92·4	66	52·8
15	♂	...	...	...	...	61·6	114·4	92·4	57·2	52·8
16	♂	...	...	...	...	48·4	114·4	88	57·2	...
17	♂	...	...	...	...	57·2	118·8	92·4	66	...
18	♂	...	...	...	...	52·8	110	92·4	57·2	48·4
19	♀	1·672	1 and 1	74·8	26·4	...	...	...	...	...
20	♀	1·292	1 and 2	70·4	26·4	...	...	...	...	...
21	♀	1·615	3 and 1	77	26·4	...	...	...	...	...
22	♂	...	...	...	...	61·6	110	83·6	52 8	48·4
23	♂	...	...	...	...	57·2	110	83·6	52·8	52·8
24	♂	...	...	...	...	48·4	101·2	79·2	52·8	52·8
25	♂	...	...	...	...	57·2	101·2	79·2	52·8	44
26	♂	...	...	...	...	52·8	96·8	74·8	52·8	...
27	♀	1·995	1 and 2	79·2	26·4	...	...	...	...	...
28	♀	1·7	1 and 1	66	26·4	...	...	...	...	...
29	♀	1·577	2 and 1	70·4	26·4	...	...	...	...	...
30	♀	1.843	2 and 1	74·8	26·4	...	...	...	...	...
31	♀	?	1 and 1	70·4	26·4	...	...	...	...	...

Nos. 1-4 are from the stomach of sheep, England.
Nos. 5-11 are from a horse, Surrey.
No. 12 from the small intestine of goat, Bromsgrove, England.
Nos. 13-21 from the stomach of horse from the Federated Malay States.
Nos. 22-31 from the fourth stomach of an ox.

The material available for examination was as follows :—

1. '*T. extenuatus.*' From the small intestine of a goat from Bromsgrove, England.
2. '*T. extenuatus.*' From the stomach of a sheep, England.
3. '*T. extenuatus.*' From the fourth stomach of an ox.
4. '*T. Axei.*' From a horse, Surrey.
5. '*T. Axei.*' From the stomach of a horse from the Federated Malay States, sent to Mr. Pillers, by Mr. G. G. B. Purvis, F.R.C.V.S., on request. (My thanks are due to both these gentlemen for this material.)

From the above-mentioned list of material a great number of worms was available for examination.

The description which follows has been found to apply to the whole of this material whether described as *T. Axei* or *T. extenuatus.*

The worms are small, fine and hair-like. The body is gradually attenuated anterior to the genital opening. Buccal cavity very small. The head is provided with three inconspicuous lips and punctiform papillae. No cervical papillae present. The head measures from 8·8μ to 13·2μ in width. The excretory pore is situated at a distance of 110μ to 176μ from the head end, and lies in a depression in the cuticle. The cuticle is transversely striated. The oesophagus is simple, measuring 514·8μ to 668·8μ in length.

The male measures, in the material examined, 2·584 mm. to 3·75 mm. in length and 44μ to 66μ in maximum breadth in front of the bursa. The bursa is comparatively well developed but there is no separate dorsal lobe. The bursal formula is as follows :—The ventro-ventral ray is thin and reaches the edge of the bursal lobe. The latero-ventral ray is as thick as the externo-lateral and medio-lateral rays, or slightly thicker. The postero-lateral ray is slightly narrower than the medio-lateral ray and is longer and thicker than the same ray in other members of the genus *Trichostrongylus* in ruminants. The externo-dorsal ray is narrow and long and its tip is about midway between the tips of the dorsal ray and postero-lateral ray. The dorsal ray is comparatively long, measuring 44μ to 52·8μ in length and is divided at its distal end into two short divisions, each of which is again bifid ; it is of about the same length as the externo-dorsal ray. The tips of the externo-lateral and medio-lateral rays are nearer together than the tips of any of the other rays.

The spicules are dark brown in colour and are very dissimilar. *The left spicule* measures 96·8μ to 123·2μ in length, and is much longer than the right one. It ends posteriorly in a thin chitinised piece which looks like a long boot, of which the toes and heels are long and sharply pointed; this appearance is best seen from the ventral view. A transverse line less chitinised than the rest of the spicule separates this part already described from the rest of the spicule roughly at about the junction of the middle and posterior thirds. There is also a narrow chitinised process directed posteriorly arising on a broad base from the dorsal surface of the spicule at about its middle. *The right spicule* measures 74·8μ to 96·8μ in length. It terminates posteriorly in a blunt and rounded end and has a less chitinised line similar to that in the left spicule, also at about the junction of the middle and posterior thirds. The spicule has a narrow and sharply pointed process directed posteriorly which originates from its dorsal side.

The gubernaculum is situated dorsal to the right spicule and measures 52·8μ to 61·6μ in length, and viewed laterally it comprises a sinuous structure with three concavities, two of which are directed ventrally; these concavities are not occupied by any weakly chitinised material as in some of the other trichostrongyles. The anterior and posterior ends are blunt. Viewed ventrally or dorsally the gubernaculum is seen to be formed of a middle swollen portion and anterior and posterior narrower portions, the swollen middle portion is asymmetrical in that it is more swollen on the right side than on the left. The anterior portion is narrower and longer than the posterior and both have rounded ends. The gubernaculum is less chitinised in the centre than at the edges.

The female measures, in the material examined, from 3·268 mm. to 4·218 mm. in length and from 44μ to 61·6μ in maximum breadth at the region of the vulva. The latter is a longitudinal crescentic slit and on account of the opening being surrounded by thick cuticular lips it appears sausage-shaped. The slit with the lips measures 44μ to 57·2μ in length and is situated at from 646μ to 792μ from the tip of the tail. The uteri are divergent and the ovejectors are not so well developed as in other species. They measure 202·4μ to 281·6μ in length. The anterior ovary bends backwards at 998·9μ to 1·995 mm. from the head end; the posterior ovary bends forwards

at 105·6μ to 215·6μ from the tip of the tail. The anus is situated at from 61·6μ to 79·2μ from the tip of the tail. The diameter of the body at the region of the anus is from 22μ to 26·4μ. The body tapers gradually and regularly from the loop of the posterior ovary to the tip of the tail; the anus is situated immediately in front of a slight elevation on the surface.

Intrauterine eggs are thin-shelled, large and elongated with the embryo inside in the morula stage. They measure from 79μ to 96·8μ by 35·2μ to 48·4μ.

Hosts infected. In the stomach and more rarely the anterior end of the small intestine of the donkey (*Equus asinus*); horse (*Equus caballus*) and in the fourth stomach and more rarely anterior end of the small intestine of cattle (*Bos taurus*); sheep (*Ovis aries*); goat (*Capra hircus*) and several other ruminants such as bharal (*Ovis nahura*); Mexican mountain sheep (*Ovis mexicana*); prong-horned antelope (*Antilocapra americana*); mule deer (*Odocoileus hemionus*); roe deer (*Capreolus capreolus*); Newfoundland caribou (*Rangifer terrænovæ*) and occasionally in man (*Homo sapiens*).

Geographical distribution. Great Britain, Europe, U.S.A., and Australia.

Discussion. The figures (1 to 11) which were drawn by the author from nature by the aid of a camera lucida, prove beyond doubt the identity of *T. Axei* and *T. extenuatus*. Figs. 1 to 5 are *T. Axei* from a horse, figs. 6 to 11 are *T. extenuatus* from a sheep. By studying the descriptions given and comparing these figures of the supposed different species, the only conclusion to be drawn is that *T. Axei* and *T. extenuatus* are identical. The slight differences in length exist, but are of no specific value as I have shown in *T. colubriformis* and *T. capricola*, etc. The name, therefore, of the parasite hitherto designated *T. Axei* and *T. extenuatus* is *T. Axei* (Cobbold, 1879) as it has the priority to the other.

SYNONYMS: *Strongylus Axei* Cobbold, 1879.
Strongylus tenuissimus Mazzanti, 1891.
Strongylus gracilis MacFadyean, 1896 (Not *S. gracilis* Leuckart, 1842).
Strongylus extenuatus (Railliet, 1898).

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THE EFFECT OF 'HÖCHST 4002' ON *TRYPANOSOMA GAMBIENSE* AND *T. EVANSI* IN MICE AND RATS

BY

E. M. LOURIE, M.B.

(*Received for publication* 30 *January*, 1931)

Giemsa (1929) describes experiments as a result of which a high therapeutic index is claimed for a new arsenical preparation, 'Arsenpräparat Höchst 4002,' the formula of which is not disclosed in his paper. For laboratory mice infected with *T. brucei* (strain 'Nagana 30'), the therapeutic index was found to be from 1 : 10 to 1 : 16, as against an index of 1 : 2 in the case of Tryparsamide and 1 : 1 in the case of Atoxyl. For mice infected with another strain of *T. brucei* (strain 'Prowazek'), the therapeutic index was 1 : 25 to 1 : 50 on subcutaneous, and from 1 : 50 to 1 : 100 on oral treatment. Other indices given by Giemsa for 'Höchst 4002' are 1 : 10 in the case of mice infected with *T. equiperdum* and 1 : 20 to 1 : 40 in the case of *T. rhodesiense*. These results being regarded by Giemsa as particularly favourable, provisional trials of the drug were, he stated, being made on five cases of human trypanosomiasis in the Cameroons, and good results were also hoped for in the treatment of trypanosomiasis of domestic animals.

In view of these promising figures, experiments were carried out at the Microbiological Institute, Hebrew University, Jerusalem, to determine the trypanocidal effect of 'Höchst 4002' on a strain of *T. gambiense* presented by Professor J. G. Thomson, of London, and also on a local strain of *T. evansi*, both in mice and in rats. Although no mention is made by Giemsa of any experiments having been carried out to test the prophylactic action of 'Höchst 4002,' the possibility of there being any such value in the drug was, however, also investigated. The writer's thanks are due to Professor S. Adler for affording facilities at the Institute for making these investigations.

'Arsenpräparat Höchst 4002' (supplied through the kindness of Messrs. Grün Bros., Tel-Aviv, Palestine) was in the form of a white crystalline powder, readily soluble in water, forming a colourless solution. The solution did not become discoloured after boiling and standing at room temperature for one month.

In all cases the drug was administered intraperitoneally, and there were no signs of local irritation following injection. The minimal lethal dose was found to be 5 grammes per kilo body weight in mice, and 8·5 grammes per kilo body weight in rats. These doses caused vertigo, and paralysis of the hind limbs. Post mortem: Small haemorrhages were found in the intestines and acute fatty degeneration of the liver.

THERAPEUTIC ACTION

The drug was tested on mice and rats infected with *T. gambiense*, the observation period after treatment being thirty-four days, and on mice and rats infected with *T. evansi*, the observation period after treatment in this case being nine weeks.

1. Mice infected with *T. gambiense*.

The results obtained by treatment of a series of mice infected with *T. gambiense* are shown in Table I.

Table I.

Showing the results of treatment with 'Höchst 4002' of mice infected with *T. gambiense*.

Mouse No.	Interval in days between infection and treatment	No. of trypanosomes per field Oc. 4 Obj. 1/6	Dose in grammes per kilo body weight	Day of disappearance of trypanosomes	Day of reappearance of trypanosomes	Remarks
12	8	1 in 2 fields	·65	1 to 2	19	Died after 25 days.
13	8	30	·7	1 to 2	19	" " 28 "
14	8	1	·8	1 to 2	23	" " 30 "
15	8	1 in 4 fields	·9	1 to 2	23	" " 30 "
16	8	35	1·0	1 to 2	23	" " 38 "
17	4	3	1·1	1 to 2	14	" " 23 "
18	4	7	1·2	1 to 2	14	" " 35 "
19	4	1	1·3	1 to 2	17	" " 34 "
20	4	4	1·4	1 to 2	17	" " 33 "
21	4	1	1·45	1 to 2	...	No relapse after 34 days.
22	4	3	1·65	1 to 2	...	" " " 34 "
23	4	4	1·8	1 to 2	18	Died after 33 days.
24	4	6	2·0	1 to 2	15	" " 18 "
25	4	4	2·2	1 to 2	...	Negative after 34 days.
26	4	4	2·4	1 to 2	...	" " 34 "
27	4	3	2·6	1	...	" " 34 "
28	4	1 in 2 fields	2·8	1	...	" " 34 "
29	4	4	3·25	1	...	" " 34 "
30	4	8	3·5	1	...	" " 34 "
31	4	1	4·0	1	...	" " 34 "

It will be seen that the minimal curative dose is 2·2 grammes per kilo body weight, and the therapeutic index 1 : 2·3. The drug does not appear to have a more marked curative effect on cases where the blood infection is comparatively light than on those cases where the blood infection is much heavier.

2. Rats infected with *T. gambiense.*

In rats infected with *T. gambiense*, doses up to 0·19 grammes per kilo body weight had no effect whatever on the trypanosomes in the blood. The minimal curative dose was 5·2 grammes per kilo body weight (during an observation period of thirty-four days), and the therapeutic index is, therefore, 1 : 1·6.

Table II is introduced to show that, as in mice, the curative action of the drug is no more marked in animals with a comparatively light infection than it is in those with a heavy infection.

Table II.

Showing the results of treatment with 'Höchst 4002' of rats infected to varying degrees of intensity with *T. gambiense.*

Rat No.	Interval in days between infection and treatment	No. of trypanosomes per field Oc. 4. Obj. 1/6	Dose in grammes per kilo body weight	Relapse, in days, after treatment
22	4	Swarming	2·0	20
23	6	4	2·3	25
24	4	1 in 7 fields	2·5	28
26	4	10	2·9	23
33	4	6	4·3	25
36	4	1 in 7 fields	4·9	31

3. Mice infected with *T. evansi.*

The curative dose in this case was found to be 1·2 grammes per kilo body weight during an observation period of nine weeks, and the therapeutic index 1 : 4·2 for the period mentioned.

4. RATS INFECTED WITH *T. evansi*.

The curative dose was found to be 1·5 grammes per kilo body weight, and the therapeutic index 1 : 5·7 during the observation period of nine weeks.

PROPHYLACTIC ACTION

In Table III will be found the results of experiments which were carried out to determine whether ' Höchst 4002 ' has a prophylactic value for mice infected with the strain of *T. gambiense* used.

TABLE III.

Showing the prophylactic effect of ' Höchst 4002 ' on mice infected with *T. gambiense*.

Mouse No.	Dose in grammes per kilo body weight	No. of days between prophylactic dose and inoculation of trypanosomes	No. of days between inoculation and first appearance of trypanosomes in blood	Result of inoculation
1	·1	3	2	Died on 12th day after inoculation.
2	·15	3	4	" " 20th " " "
3	·17	10	1	" " 7th " " "
4	·2	3	7	" " 20th " " "
5	·23	10	1	" " 7th " " "
6	·3	3	7	" " 14th " " "
7	·32	10	1	" " 17th " " "
8	1·0	3	3	" " 14th " " "
9	1·5	3	5	" " 9th " " "
10	3·0	3	1	" " 34th " " "
11	4·0	3	1	" " 71st " " "
12	4·5	3	2	Alive after 84 days.
13	4·6	12	1	Died on 15th day after inoculation.
14	4·7	3	5	" " 11th " " "
15	4·8	12	1	" " 21st " " "
16	4·9	3	7	" " 27th "

The above table shows that the drug has no prophylactic value after three days, even when administered in doses approaching the minimal lethal dose, but that the average length of life of mice inoculated three days after the injection of small doses of the drug was twice that of mice inoculated ten to twelve days after giving the drug, and the incubation period was longer in the former than in the latter.

In the case of infections with *T. evansi* in mice, the drug given in doses of from 0·6 to 5 grammes per kilo body weight had no prophylactic action after six days, the only effect being to increase the incubation period from 1 to 2 days in control mice up to 8 to 14 days in treated animals.

In rats, doses of from 0·5 grammes to 5 grammes per kilo body weight had no prophylactic action against *T. evansi* infection when trypanosomes were inoculated six days after administration of the drug, and no increase in the incubation period was observed as compared with that in control animals.

CONCLUSION

The values obtained in the writer's series of therapeutic experiments with ' Höchst 4002 ' are all very considerably less favourable than those quoted by Giemsa ; and it is interesting, in this conclusion, to note that disappointing results have also been recorded by Nieschulz and Wawo-Roentoe (1930), not only in experiments on mice, rats and guinea-pigs infected with a strain of *T. evansi*, but also in the case of similarly infected horses.

The prophylactic value of ' Höchst 4002 ' as judged by the writer's experiments, is negligible.

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NOTES ON AFRICAN ANOPHELINES

BY

A. M. EVANS

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I. FURTHER NOTES ON THE *MARSHALLI* GROUP OF *ANOPHELES*

In a previous paper (Evans, 1929) the larva of *A. marshalli* var. *moucheti* and pupa of this form and also of the varieties *hargreavesi* and *freetownensis* were described, as well as the male hypopygium of *moucheti* and *freetownensis*. More recently, Dr. Barber and Mr. Olinger, of the West African Yellow Fever Commission of the Rockefeller Foundation, have obtained all the stages, including the eggs, of *hargreavesi* and also of a form of *moucheti* differing from the type chiefly in a larval character. From a study of this most interesting material, it appears that at least two of these forms, namely *A. moucheti* and *A. hargreavesi*, should be regarded as distinct species. This conclusion is further strengthened by an examination of a series of *hargreavesi*, including three males, received from Dr. H. P. Fowler, at Ibadan, Southern Nigeria, and a good series of *moucheti*, including larvae and pupae reared from eggs obtained by Mr. E. G. Gibbins, in Uganda. I have also been able, through the kindness of Dr. P. A. Buxton and Mr. H. S. Leeson, to examine the male hypopygium of the type form of *marshalli* from Southern Rhodesia. Other material dealt with in this paper is an interesting collection of specimens of the *marshalli* group made by Mr. Symes in several different localities in Kenya. Amongst this latter collection are a series of females and one male which are here described as a new variety of *marshalli*.

ANOPHELES MARSHALLI Theobald, type form

Male terminalia. Phallosome with about five leaflets on each side arranged as shown in fig. 2, B. Longest leaflets somewhat shorter and broader than those of *moucheti* but narrower, on the whole, than in

freetownensis. Parabasal spines of coxite (side-piece) five in number. Lobe at base of coxite with club less sharply dilated than in *hargreavesi* and with one accessory hair present, rather external to the apical bristle.

ANOPHELES MARSHALLI var. *KENIENSIS* n. var.

This variety differs from the type form from Southern Rhodesia as follows :—

Wings without pale interruption in third dark spot on first longitudinal vein (R1). Outstanding scales of wings (fig. 1, B), considerably shorter and broader than in typical *marshalli* or var. *pitchfordi*. (It should be noted that, in examining these scales, due allowance must be made for the fact that the outstanding scales on the stems of the second and fourth veins appear much narrower than they really are, when turned over into an ' edge on ' position, as frequently happens.) Mesonotal scales resembling those of *marshalli* on anterior half but, on posterior half, very narrow, almost hair-like, and scattered among numerous bristles.

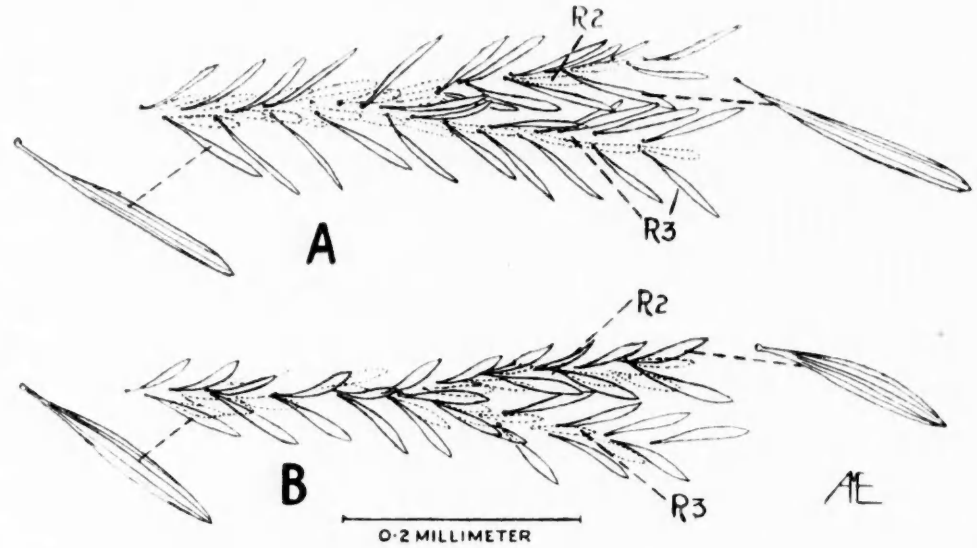

FIG. 1. Part of upper surface of stem and branches of second vein (R2 and R3). *A.—A. marshalli* Theo., type form ; *B.—A. marshalli* var. *keniensis* n.var.

The variety also shows a great tendency to darkening of the wing veins, especially the second, fourth and sixth and basal three-fifths (approximately), of the costa, which is uninterruptedly dark in four of the nine specimens, and shows only incomplete interruptions in two others. The hind tarsi show a marked tendency to reduction of the pale rings which are usually very narrow and indistinct and

may be practically absent on all the segments or distinct on the first two segments only.

Male terminalia. The characters of the hypopygium of the one male of this variety examined are as follows :—parabasal spines five in number ; lobes of coxites with an inner accessory bristle in addition to the usual outer one. Phallosome with five leaflets, the four shorter ones appearing somewhat narrower than in the specimens of typical *marshalli* examined ; relative lengths of the leaflets rather different from the condition in *marshalli,* the two next in length to the longest leaflet being almost equal to each other and the next one approaching these in length (fig. 2, C). The character of the leaflets is markedly different from that of *freetownensis* (see Evans, 1929), which this variety approaches in certain external characters.

Kenya : Co-types 2 ♀♀, Jesereo, 17 January ; 2 ♀♀, Simba, 20 January ; 1 ♂, 20 January. Also 2 ♀♀ taken Simba, 20 and 31 January, and 2 other ♀♀.

All specimens taken in 1930, by Mr. C. B. Symes.

ANOPHELES MARSHALLI var. *PITCHFORDI* Giles

Several females provisionally referred to this variety were collected in 1930, at Kakamega and Kiambu, in Kenya, by Mr. Symes. These specimens differ slightly from the type series of *pitchfordi* in possessing a patch of narrow whitish scales above the wing base which, in some of the specimens, extended forwards almost to the fossae. Mr. Edwards, who kindly examined the original series of *pitchfordi* agreed that in other respects the Kenya specimens resembled that variety.

ANOPHELES HARGREAVESI Evans

Anopheles marshalli var. *hargreavesi* Evans, 1927 and 1929

As this form is morphologically distinct in the larval, pupal and male characters from the type form of *marshalli* and also from all allied forms, in which these characters are known, it is here considered to be a distinct species. In its most obvious external character, the broad scales extending to the posterior border of the mesonotum, it resembles *A. marshalli* var. *austeni,* the morphological characters

of which are undescribed. The latter form, however, differs from *hargreavesi* at least in the much wider banding of the front tarsi, which is basal as well as apical, in the possession of a well-marked patch of broad scales extending forward from the above wing root at the extreme sides of the mesonotum (Mr. Edwards kindly informed me of the presence of this character in the type of *austeni*), and also its much larger size. Mr. Edwards tells me that the wing of the type measures 3·9 mm., and the body, without proboscis, about 4·2 mm. In *A. hargreavesi* the corresponding measurements are *c.* 1·8 mm. to 3·0 mm. and *c.* 2·0 mm. to 3·3 mm.

Now that Dr. Barber's series of Southern Nigerian specimens and those of Dr. Fowler, both including males, have been examined, it is possible to give a more comprehensive description of the form than those hitherto published.

FEMALE. Palps normally with the broad apical bands separated by a dark band which is narrower than either pale band; sub-apical pale band, in some specimens, considerably shorter than apical and not appreciably longer than dark band. Wings with light and dark areas rather variable in extent. Costa with four main dark areas and usually with two pale interruptions of variable size on the basal third. Pale area proximal to third main dark area sometimes very small (about one-third of dark area); in other specimens larger (half or more than half the length of the dark area). Pale area beyond third dark area also variable, in some cases greater, in others less than the fourth main dark area. First vein with dark areas under the four main dark ones of the costa; third dark area interrupted by pale spot (sometimes very small) which is usually at about the middle of the area but in one wing of the type it is continuous with the proximal pale area. Second dark area with accessory sector spot present and, either continuous with proximal pale area, or separated from this by a dark spot. Second vein with either or both branches often extensively dark, stem with two dark areas of variable extent. Third vein pale with small apical and sub-basal dark spots (an additional one is occasionally present at the base). Fourth vein usually with two dark areas on upper branch and one on lower; stem mainly pale but with dark area before fork and usually one or two more proximally. Fifth vein usually mainly pale with two or three dark spots on upper

branch, one or two on lower branch and two on the stem, one of these often continuous with dark areas on the branches. Sixth vein usually with three dark spots. Fringe with pale spots at apices of third, branches of fourth and fifth and usually sixth veins.

Mesonotum clothed with mostly broad, greyish-white scales (index about 4 to 6, see Evans, 1927, fig. 8, A) some of which extend back to the scutellum; a large proportion often truncate.

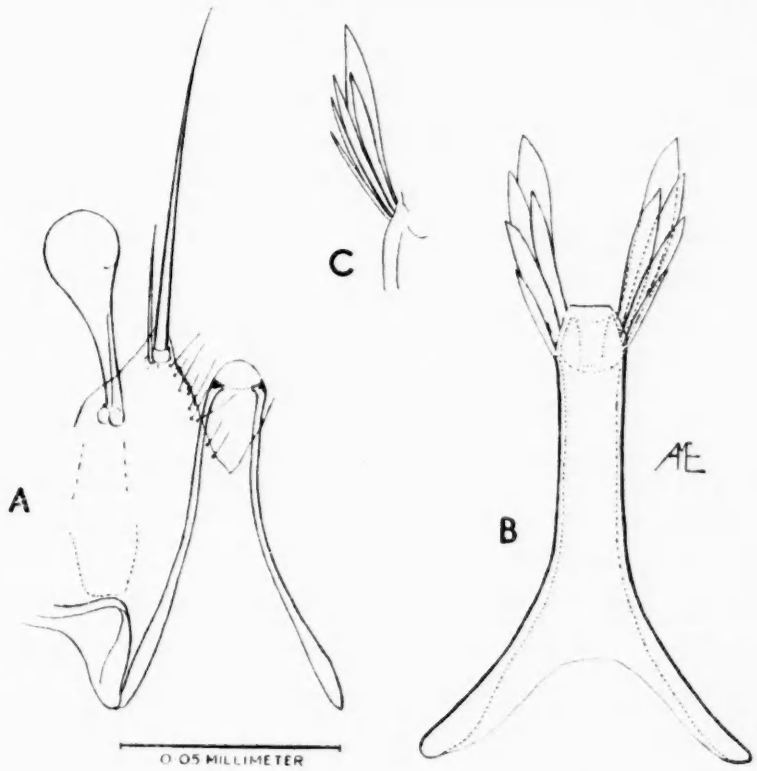

FIG. 2. ♂ terminalia. *A.*—*A. hargreavesi* Evans, phallosome and basal lobe of one coxite; *B.*—*A. marshalli* Theo., type form, phallosome; *C.*—*A. marshalli* var. *keniensis* n.var., tip of phallosome with leaflets of one side. All figs. to same scale.

Front tarsi with well-marked apical pale rings which are often moderately broad (length about one and a half times to twice the apical width of the segment) on first two segments; narrower on the third and often very minute on fourth. Mid tarsi usually with moderately broad apical rings. First four segments of hind tarsi with apical rings which are usually narrow, those on the first two segments normally about equal to the apical width of the segments.

Size. Wing length *c.* 1·8 mm. to 3·0 mm.

MALE. Club of palp with a large apical whitish patch, dorsally, on each segment and with a narrow pale ring at the base.

Terminalia (fig. 2, A). This differs from that of other members

of the *marshalli* group, in which the structure is known, in having the phallosome entirely without leaflets. The parabasal spines are normally five in number, but in one specimen a supernumerary one was present. Ventral lobe of coxite with one fine accessory hair in addition to the apical bristle ; club with extremity sharply dilated so that it usually appears almost globular. Phallosome entirely devoid of leaflets, the tubular portion appearing shorter than in *moucheti* or *marshalli* var. *freetownensis*, but this may be due to the fact that the apex is directed dorsally, resulting in considerable foreshortening when viewed as in fig. 2, A.

Fourth stage larva. This small larva closely resembles that of *A. moucheti* var. *nigeriensis*, but differs chiefly in the size of the abdominal palmate bristles.

Inner clypeal hairs (fig. 3, B) long and simple ; outer simple and about two-fifths the length of the inner ; posterior short and simple. Mental plate, thoracic palmate bristle and anterior submedian thoracic bristles very much as in *moucheti* (see Evans, 1929), but the latter with their bases sometimes separated. Pleural hairs as in *moucheti*, the long bristles agreeing with those of ' Group I, Division B ' of Puri (1928).

Abdominal tergal plates very slightly larger, on the whole, than in *moucheti*. First to seventh segments with palmate bristles, those on first segment small and closely resembling those of *moucheti*. Typical palmate bristles distinctly smaller than in *moucheti*, that on fifth segment (fig. 3, C) with leaflets measuring *c.* ·06 mm. ; while on the corresponding segment in *moucheti* the length is *c.* 0·8 mm. to 0·9 mm. The filaments of the leaflets are relatively shorter than in *moucheti*, the ratio of length of filament to total length of leaflet being about 1 : 3 to 1 : 4 or more in *hargreavesi* and *c.* 1 : 2·4 to 1 : 2·9 in *moucheti*. Lateral comb with long and short teeth somewhat variable in number and arrangement ; not distinguishable from that of *moucheti*.

Pupa. No marked differences were observed between Dr. Barber's pupal pelts and the one collected by Dr. Schwetz, near Stanleyville. In my description of that specimen, however, it was noted that the respiratory trumpet appeared to possess a short meatus and, while this is the case in the majority of the Nigerian specimens, in one or two of them the opening was continued

practically to the base of the trumpet. A further small variation concerns the number of branches in the sublateral bristle on the fifth abdominal segment which had four branches in one of the specimens so that the number varies from two to four.

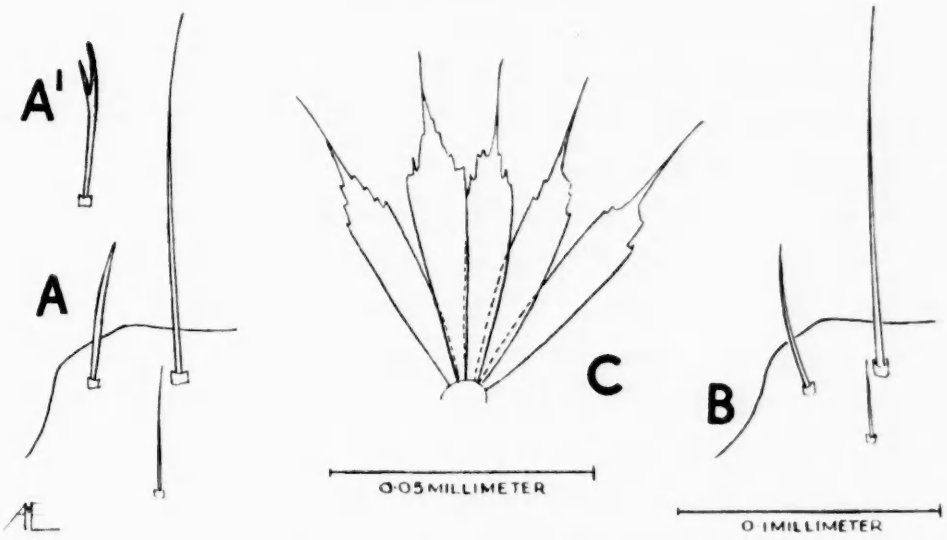

FIG. 3. *A*.—Clypeal bristles of *A. moucheti* var. *nigeriensis* n.var.; A^1.—Outer clypeal hair of same, atypical. *B*.—Clypeal bristles of *A. hargreavesi*; *C*.—A few leaflets from median part of palmate bristle of fifth abdominal segment of *A. hargreavesi*; *A* and *B* to same scale.

Eggs. Dr. Barber and Mr. Olinger have studied the eggs of this form in both the immature and mature condition and have discovered that they show well-marked characteristics. The egg is of the usual boat-shaped form, with a pair of large dorso-lateral floats each showing about 14 to 18 major striations. It also bears, at each

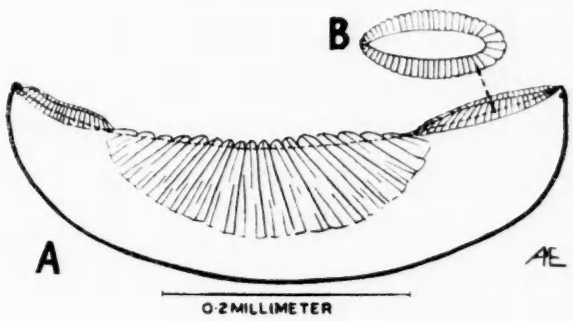

FIG. 4. *A*.—Mature egg of *A. hargreavesi*, lateral view; *B*.—Anterior frill, dorsal view.

pole, on the dorsal surface, a smaller, oval frill, as shown in fig. 4. These frills do not project markedly from the surface and are most obvious when the egg is viewed from the dorsal surface. They are

conspicuous in immature eggs and Dr. Barber tells me that he finds them of considerable use in identifying ♀♀ dissected to determine the infectivity.

It should be noted, however, that Dr. Barber dissected out immature eggs without well-developed frills from at least two females of *A. hargreavesi*. These eggs, appear to be somewhat younger than the immature eggs in which the frills are developed, and it seems possible that these structures may be still undifferentiated in these apparently aberrant specimens.

Described from specimens collected within 15 miles of Lagos, 1930, by Dr. Barber and Mr. Olinger :—Three larval and pupal pelts, Ebute Metta, 5 September ; three larval and pupal pelts, Otta, 1 and 2 October ; also a larval and pupal pelt, Jebba St., 13 September, and several larvae collected at Jebba Pool and immature and mature eggs obtained from ♀♀.

Breeding places. Dr. Barber informs me that he and Mr. Olinger have found larvae of this species in the following types of water :— (1) foul, sewage-contaminated water covered with *Pistia*, both in the sun and in partially shaded places ; (2) in *Pistia* in clear water in more open jungle areas ; (3) in grass growing in open swamps.

ANOPHELES MOUCHETI Evans

Anopheles marshalli var. *moucheti* Evans, 1925, 1927 and 1929.

The discovery by Dr. Barber of the larva of *A. hargreavesi* shows that that form and *A. moucheti* are rather closely related in larval characters but, as there are distinct differences in this stage and very marked differences in all the other stages, there seems to be no doubt that the two are distinct species. The larva, moreover, differs markedly from those of the type form of *marshalli* and var. *freetownensis*, and the adults differ in wing markings and hypopygial characters from both these forms and in wing and tarsal markings from var. *domicolus* so that there seems no doubt that it is specifically distinct. Dr. Barber has also discovered the early stages of a form of *moucheti* occurring near Lagos, differing in a larval character and also, to some extent, in wing markings, from the type form of *moucheti*. The specimens with typical larvae collected in Uganda by Mr. Gibbins agreed closely with the Congo form in all adult and larval characters.

A short diagnosis of the adult characters of the type form of the species is given below :—

FEMALE. Palps with two very broad equal or subequal white bands distally, one involving the apex and a narrow proximal white ring ; distal bands separated by a dark ring which is usually less than the pale bands and often about half the length of these ; it may be considerably narrower than this and in two specimens seen it was absent.

Wings. White areas of costal region reduced and third main dark area very long, relative lengths of the light and dark areas usually about as shown in the illustration of a co-type wing (Evans, 1925 and 1927), but a few specimens show a slight lengthening of the pale areas on the outer half of the costa. No pale interruption in third main dark area on first vein. Third vein mainly pale with two small dark areas at and near the base and a larger one at the apex ; the sub-basal dark area, in a few specimens, about twice as long as shown in my figure. Other veins with dark and light areas much as illustrated for the co-type, the lower branch of the second vein being mainly dark and upper branch lighter in nearly all the specimens ; stem of fourth vein sometimes darker and sixth vein sometimes with dark areas more extensive, especially on outer half, which may be entirely dark. Fringe spot at end of sixth vein usually absent, rest much as in figure of co-type (Evans, 1927). Outstanding scales of wing shorter and broader than in the type form of *marshalli*.

Mesonotum with scales on anterior half either broad (index 4 to 6) with many scales truncate, or rather narrow (index 6 to 8) and all finely pointed (all the Uganda specimens and some of those from Stanleyville) ; on posterior half, scales mainly very narrow (about as in *funestus*), but a few rather broader ones sometimes present laterally.

Legs. Tibiae and first three tarsal segments of front legs and first four of mid and hind legs with apical whitish rings ; those on front tarsi generally slightly wider than on hind tarsi, where they are about equal to the apical width of the segment.

MALE. Palps as in *A. hargreavesi*.

Terminalia. Phallosome with about four narrow leaflets on each side (see Evans, 1929).

Size. About as *funestus.*

Distribution. Belgian Congo, various localities; Uganda: Jinja (Mr. E. G. Gibbins).

Breeding places. Schwetz (1930) records larvae of this species from amongst weeds at the edges of streams and ponds sometimes in association with *funestus, nili* and *mauritianus.* In a previous paper (1929), I stated that Dr. Schwetz had found larvae in ' ruisseaux, herbes a sol ' ; this should have been ' herbes à sel ' which, Dr. Schwetz informs me means ' salt grass,' a plant from which the natives make salt.

A. MOUCHETI var. *NIGERIENSIS* n.var.

This variety differs from the type form as follows:—Wing markings more variable ; upper branch of second vein usually much darker, often with only a very small pale spot ; lower branch frequently much paler than in type form ; third vein with apical dark area much shorter and sub-basal dark area usually longer than in typical specimens of the type form. The scales on the anterior half of the mesonotum are of the narrower type seen in *moucheti* and, as usual, those on the posterior half are narrower still. Leaflets of phallosome exactly as in *moucheti.*

Larva. The larva differs from that of the type form in the character of the outer clypeal bristles (fig. 3, A), which are simple or, occasionally bifid, but in the type form show at least four branches. Whether simple or bifid these bristles appear rather blunt pointed, their extremities not being drawn out into long fine points.

Pupa. The pupa resembles the type form in the main features of the chaetotaxy, but the trumpet seems to differ slightly in that the opening does not reach to the base as is the case in *moucheti,* but this character is often a difficult one to determine exactly.

Eggs. As Dr. Barber has observed, this egg differs from that of *A. hargreavesi* (fig. 4) in the absence of well-developed terminal frills. In the positions where these occur in *hargreavesi,* very rudimentary frills are present which are so slightly developed as to appear as oval lines when viewed from above, even with the one-sixth objective. As Dr. Barber also notes, the ' network which is formed by the outer layer of the eggs, is relatively coarse as in *funestus.*'

Southern Nigeria. Co-types, 2♀♀ Yaba, 29 September and 4 October; 1♀ and 2♂♂ reared from larvae, Ebute Metta, near Lagos and larval and pupal pelts of these. Several larvae taken at Ebute Metta and mature and immature eggs from ♀♀, Yaba. All specimens and 4♀♀ (dissected and mounted on slides) collected by Dr. Barber and Mr. Olinger, 1930. 1♀, Lagos, November, 1928, Mr. C. B. Philip.

Breeding places. Dr. Barber and Mr. Olinger found numbers of larvae of this variety in clear, grassy water near Railway shops, Ebute Metta, and also in clear water with *Pistia* and other vegetation, as well as among grass growing in open swamps with clear water.

II. THE EARLY STAGES OF *ANOPHELES OBSCURUS* Grünb.

Larvae of this species have been collected by Dr. Barber and Mr. Olinger, near Lagos, and adults reared from isolated specimens. I find that the hypopygium of a male reared by Dr. Barber agrees with that of *obscurus* in having the very large leaflets as discovered by Christophers. In addition, four pupal pelts, collected in Uganda, which agree with that taken near Lagos, have been received from Mr. Hopkins. Dr. Barber and Mr. Olinger also obtained eggs of this species which are described below.

Fourth stage larva (fig. 5). Inner clypeal bristles long and simple; outer dendroid but with relatively few (about 10 to 20) branches, clypeal bristles very short and apparently simple. Antenna with well-developed spicules; hair on shaft arising well before middle, sometimes at about one-third of the distance from the base; hair at apex of antenna with several branches, many of which divide again so that numerous ultimate branches (18 to 25) are formed, the branches are often seen foreshortened in whole specimens of larvae, so that the appearance differs from that shown in fig. 5, A. Posterior frontal and vertical bristles (see Patton and Evans, 1929, p. 242), relatively large, with about 8 to 10 branches. Mental plate with teeth very uneven, those nearest to the apical tooth much smaller than the next teeth, their apices not reaching as far, distally, as those of the latter. In this character they resemble the larva of *moucheti* (see Evans, 1929, fig. 1, B) but the sub-apical teeth are even shorter, relatively than in that form.

Inner anterior submedian thoracic bristles relatively well developed, with about ten branches ; middle ones with about twelve branches (fig. 5, D). Thoracic palmate bristles with about ten narrow, pointed, shoulderless leaflets. Pleural bristles showing the main characters of the sub-genus *Anopheles*, but posterior

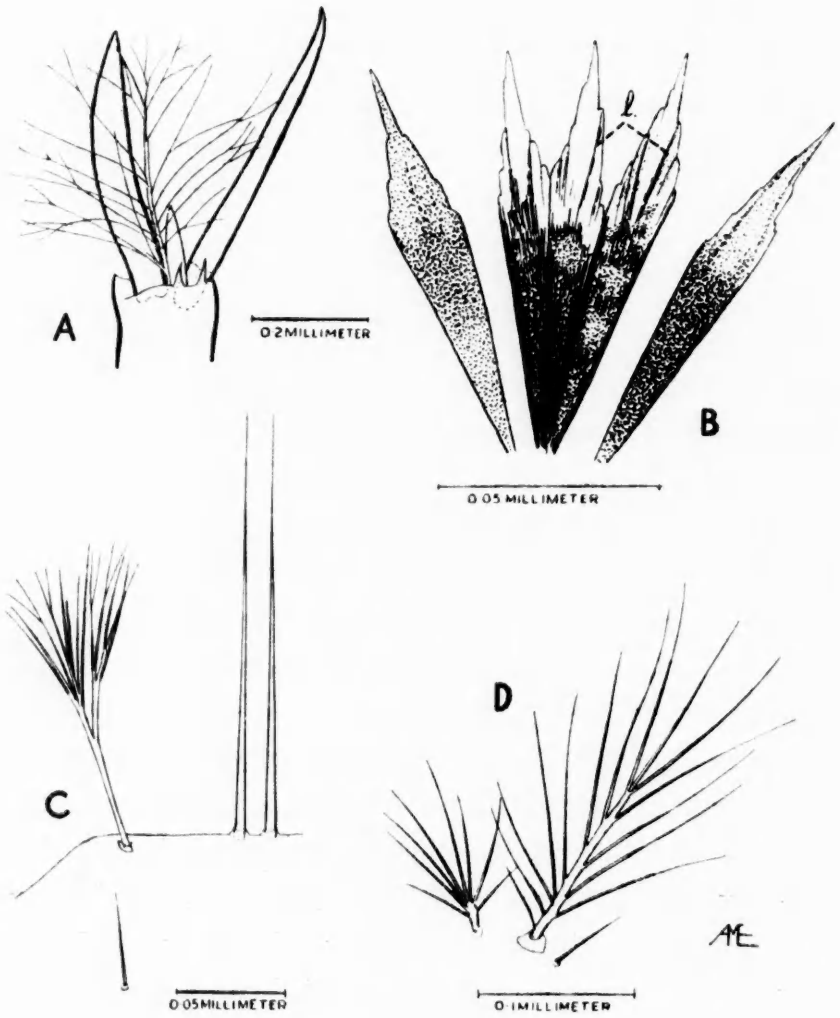

FIG. 5. *A. obscurus* Grünb., larva. *A.*—Tip of antenna showing branched hair (The measurement of the scale should be ·04 millimeter); *B.*—Leaflets of palmate bristles of fifth abdominal segment showing variation in markings in three different specimens; *C.*—Clypeal bristles; *D.*—Right anterior submedian thoracic bristles.

ventral bristle of metathorax usually divided into three branches. Thoracic clinging organ, ' contractile thoracic appendage ' of Iyenger, 1928, very large and prominent in most of the specimens, the membraneous portion, ' cuticular wing,' much less delicate than in other species such as *mauritianus* and *sinensis* and often showing

brown pigmentation which is strongly developed in some examples. Lateral plumose bristles on first three abdominal segments well developed. First and second abdominal segments with rudimentary palmate bristles very similar to those on metathorax, that on second segment larger than that on first. Palmate bristles of third to seventh abdominal segments well developed, leaflets usually pigmented, but those on sixth and seventh segments sometimes colourless. Leaflets very similar to those of *mauritianus*, but distal part with serrations rather less angular. Outer portion clear or less deeply pigmented than basal half, and often showing an appearance of fine lines (as shown in fig. 5, B, *l.*). Lateral comb with about nine long teeth, alternating in most cases with a single short tooth.

This species was formerly thought to be identical with the Oriental *A. umbrosus* but, by an examination of the male hypopygium Christophers demonstrated that it was a distinct species. This conclusion is amply supported by the characters of the larva which, as Dr. Barber noted, differs markedly from that of *A. umbrosus* in possessing well-developed palmate bristles The branching of the outer clypeal bristles resembles that of *umbrosus* more than that of *mauritianus*, but there are fewer branches in *umbrosus*, usually only six to eight. Other marked differences are the larger hair on the shaft of the antenna, with more numerous branches in *obscurus* ; the much greater number of branches of the apical antennal hair (18 to 25 in *obscurus*, but only about five or six in *umbrosus*) ; the larger and more branched posterior frontal and vertical bristles and the well-developed inner submedian thoracic bristle of *obscurus* which, in *umbrosus*, is very small and shows only two or three branches.

The differences from the larva of *A. mauritianus* are not quite so marked, but the outer clypeal bristles are very much less branched than in that species in which the ultimate branches may be fifty or more. The lateral comb shows only one small tooth between successive long teeth in *obscurus*, whereas in *A. mauritianus* there are two or three short teeth between at least some of the adjacent long teeth ; this character was first noticed by Mr. E. G. Gibbins. Further distinct differences are that the branched hair on the shaft of the antenna in *mauritianus* has less and shorter branches than in *obscurus*,

and the apical antennal hair also shows many less branches (10 to 12) in *mauritianus*. The well-developed inner submedian thoracic bristle of *obscurus* also differentiates it from *mauritianus* in which this is very short and three- to five-branched. The mental plate of *mauritianus* differs in having the teeth of regular size, much as in *A. marshalli* var. *freetownensis* (see Evans, 1929, fig. 1, c). The leaflets of the palmate bristles have the serrations more angular in *mauritianus* than in the larvae here described.

Pupa. Since going to press a further collection of material has been sent by Dr. Barber, including three pupal pelts of *obscurus* which show marked differences from those examined before. The description of this pupa has, therefore, been withdrawn from the present paper and will be included in a later publication dealing with the pupae of several species of mosquitos recently received from Mr. Hopkins.

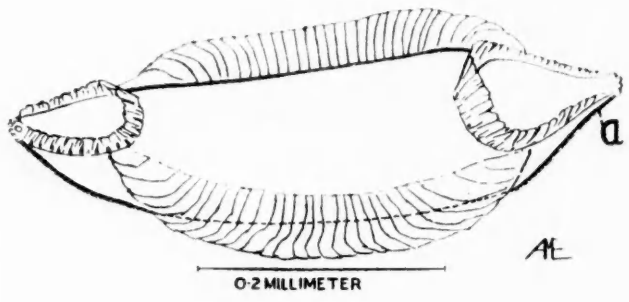

FIG. 6. *A. obscurus.* Egg, dorsal and slightly lateral view; *a.*—anterior end.

Eggs (fig. 6). The egg, as usual, is boat-shaped and widest at the anterior end, but both ends are produced dorsally into a narrow rounded projection. The dorso-lateral floats are very long and show about 27 striae. Dorsally there is a conspicuous frill at each pole, enclosing a roughly triangular area. The frills project at right-angles to the surface, and the anterior one, which is the longer, is of considerable length proximally.

Described from twelve larvae and a larval pelt taken in a ditch, adjoining a cocoa tree nursery in woods, and also several eggs from ♀♀, near Lagos, 1930, Dr. Barber and Mr. Olinger

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MISCELLANEA

TRYPANOSOMA RHODESIENSE IN THE CEREBROSPINAL FLUID OF SHEEP AND GOATS

(*Received for publication* 6 *January*, 1931)

In the late stage of infection of sheep and goats with *T. rhodesiense* the animals show nervous symptoms. There are incoordinated movements of the head; sometimes an animal will walk round in circles or wander about aimlessly apart from the others, and not infrequently convulsions occur. Three goats and two sheep were chloroformed to death and cerebro-spinal fluid withdrawn by suboccipital puncture after dissection, and examined with a Fuchs and Rosenthal counting chamber with these results :—

Animal.	Living *T. rhodesiense*.	Cells.
Sheep 96... ...	70 per cmm.	4,800 per cmm.
,, 78... ...	79 ,,	Not counted.
Goat 94... ...	77 ,,	257 per cmm.
,, 97... ...	6 ,,	297 ,,
,, 100... ...	1 ,,	207 ,,

When trypanosomes were numerous, they rapidly became attached by the posterior end to the larger leucocytes.

J. F. CORSON.

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ANNALS

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IN MEMORIAM

HAROLD WOLFERSTAN THOMAS, M.D., C.M.

Died at Manáos, 8 May, 1931

The Editors regret to announce the death of Dr. HAROLD WOLFERSTAN THOMAS, who has been closely associated with the Liverpool School of Tropical Medicine for the past twenty-eight years.

In 1903 he was appointed Director of the Runcorn Research Laboratory where he devoted himself especially to the study of experimental trypanosomiasis. It was during this work that he discovered the value of atoxyl in the treatment of trypanosomiasis. This discovery, although empirical, proved to be of fundamental importance in the subject of chemotherapy.

Dr. THOMAS was a member of the 15th and 16th Expeditions sent by the Liverpool School of Tropical Medicine to Brazil in 1905 and 1910, respectively, to investigate Yellow Fever. He established a scientific laboratory in 1905 and spent practically the whole of the last twenty-five years as its Director.

HAROLD WOLFERSTAN THOMAS

STUDIES ON THE LIFE-HISTORY OF *TERNIDENS DEMINUTUS*, A NEMATODE PARASITE OF MAN, WITH OBSERVATIONS ON ITS INCIDENCE IN CERTAIN REGIONS OF SOUTHERN AFRICA

BY

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(*Received for publication* 30 *December*, 1930)

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HISTORICAL INTRODUCTION

The history of our knowledge of *Ternidens deminutus* has been a very discontinuous one.

The parasite appears first to have been collected by Monestier, a physician in the French navy who, in 1865, collected specimens at a post-mortem examination made in the Cormoro Islands, on a native of the Makona tribe (probably from the Makona region of Mozambique), and referred to them as *Ancylostoma duodenale*. Represented by a single pair of nematodes, it was not until forty years later that Railliet and Henry (1905) found it in a vial, carrying the label ' Intestinal worms recovered at autopsy from an African native at Mayotte,' which had been deposited by Monestier in the natural history museum in Paris. These authors, differentiating

the parasite from the other worms in the vial, described it as *Triodontophorus deminutus*, a new sclerostome parasite of man. When, in 1909, the taxonomy of the nematode family Strongylidae was subjected to a revision by Railliet and Henry, the new human parasite was differentiated generically from the equine species of Triodontophorus and the new genus Ternidens was established for its reception. This genus is still represented by the solitary species, *T. deminutus*.

The second record of the finding of *Ternidens deminutus* was not made until 1908, when Turner found a few female worms in the large intestine of a Nyasaland native who had died on the mines in Johannesburg. Provisionally distinguishing the worms by their size and unusual habitat from the examples of *Necator americanus* that were found in the small intestine, Turner referred the material for identification to London. Leiper (1908) re-examined the worms and found them to agree in all major particulars with Railliet and Henry's description of *T. deminutus*. He considered the new record to be of special interest as indicative of a somewhat wide geographical distribution of the parasite in Africa.

No other specific records of the occurrence of the parasite in man are reported in the literature. In Brumpt's well-known ' Précis de Parasitologie ' (1927, 4th ed., p. 676) there is to be found a summary statement of Brumpt's finding of *Ternidens deminutus* in the Asiatic monkey, *Macacus sinicus*, and of its having been found by Noc and Barrois in *Macacus cynomolgus*.

Manson-Bahr, referring to Ternidens in the most recent (9th) edition of ' Manson's Tropical Diseases,' makes the following statement : ' This is a not uncommon parasite of man in the Transvaal and Nyasaland, but, unless it occurs in large numbers is of no pathological importance.' In the previous (7th) edition (1921), the statement is made that Ternidens ' has several times been found in natives post-mortem by Turner, in the Transvaal, and by Stannus, in Nyasaland.' From a personal conversation with Dr. Manson-Bahr on this subject, it appears that the information upon which the above statements were made was drawn from Turner's post mortem finding of 1908 and some material collected by Dr. Stannus. From communication with Dr. Hugh S. Stannus it was learned that material subsequently identified by Leiper was secured by Stannus,

either from the stools or recovered post-mortem from prisoners at Loma, Nyasaland.

In no case has the infection been diagnosed from the eggs in the stools of the patient, and in none of the recent annual reports of the various Government medical research laboratories in East or Central Africa is mention made of the occurrence of Ternidens infections.

It is apparent from what has been written above that the records of Ternidens infestation are perhaps as meagre as they are for any of the rare or little-known parasites of man. It has been suggested from the available evidence that Ternidens occurs probably accidentally in man, its normal host being monkeys. On the other hand, the statements already cited in Manson's classical text would put an entirely different complexion on the question and, if it be true, we are forced to wonder why no further reports on this subject have been forthcoming.

The writer's interest in the subject began in 1928, when he was confronted with the case of a medical missionary, long resident in Southern Africa, whose stools presented eggs that had previously been identified as those of the hookworm. Convinced that this diagnosis was inaccurate, a study of the parasite was undertaken with a view to establishing the correct diagnosis. Careful observations were made on the eggs and on the morphology of the larvae hatched from these eggs. To determine the identity of the worm, infective larvae were fed experimentally to rabbits and a monkey without success. Subsequently the successful infection of man was obtained but all efforts to secure adult worms by anthelminthic treatments were of no avail. Consequently, in our paper on this subject (Sandground, 1929) it was necessary to record the infection as due to a specifically unidentified parasite. The suggestion was put forth, however, that because of a general resemblance in size and shape of the eggs with those of certain species of trichostrongyles which have long been known to occur as occasional parasites in man, our parasite might be a member of this group but there were at our disposal no diagrams of larvae adequate enough to warrant the indictment of any particular species of this large group.*

*From later comparative studies of the larvae of trichostrongyles of sheep in Rhodesia, we are now of the opinion that the parasite whose eggs and larvae were described and illustrated in our 1929 paper is possibly *Trichostrongyles instabilis*, already recorded several times from man. Final proof of this is still, unfortunately, lacking.

During the process of our investigations, a request was sent to the Mission in Rhodesia, where it was believed our patient had probably contracted his infection, for some worms that had been passed by a native following hookworm treatment and that had been identified locally as *Necator americanus*. In reply to this request, seven worms, all of which proved to be *Ternidens deminutus*, were received together with a sample of the faeces of another native infected with what was considered to be the same parasite. The female worms received were unfortunately non-gravid and we were thus deprived of the much desired opportunity of examining their eggs to control our diagnosis.

The sample of faecal material that accompanied the worms was apparently from a very light infection for it was only after prolonged search that a few nematode eggs were found. These measured from 83μ to 86·4μ in length, by 43·2μ to 47μ in breadth, and conformed generally in shape with those that had been engaging our attention. These eggs were said to be the usual forms encountered in the stools of a large proportion of the local native population. Subsequently a second lot of worms—mainly Ternidens and a few Necator—were received from this mission.

As is apparent from the account detailed above, the situation was an involved one. It was evident that Ternidens was not uncommon in the region but, in order to determine the incidence of the infection and to study its epidemiology and the unknown features of its life history, investigations in South Africa were necessary. A portion of the accumulated interest of a fund very generously bequeathed by the late Dr. Frederick C. Shattuck, to the Department of Tropical Medicine in the Harvard Medical School, was made available by Professor R. P. Strong for this field work and in January, 1930, the investigations described in the following pages were commenced.

Thanks to the interest of Dr. A. J. Orenstein, Chief of the Sanitation of the Rand Mines, Ltd., and of Dr. W. A. Fischer, in charge of special investigations, an opportunity was provided to work in the native hospital of the City Deep Gold Mine in Johannesburg. The very large native labour force on the mines present a unique and exceptionally convenient opportunity for studying the parasitic infections of South African aborigines without having to travel

extensively in the country. Certain facilities for post-mortem examinations were also available at this hospital.

After working at this centre, our next research station for ten weeks was at Mount Silinda, situated on latitude 19° S. in the Melsetter district on the south-eastern border of Southern Rhodesia. Mount Silinda is the principal seat of activities of the American Board of Foreign Missions in Rhodesia. The natives of the region are of the Ndau tribe, a branch of the Shangaan nation. In addition to serving the local population, the mission also attracts natives from a wide area to its school and industrial departments. It was from Mount Silinda that our original parasite material was derived, and from this station the medical needs of the scattered native population and white settlers are so capably and nobly served by Doctors W. T. Lawrence and W. L. Thompson. To these gentlemen, their wives and co-workers at the mission, who, holding the confidence and high esteem of the population, made work with the natives possible, I am deeply indebted. Examinations were also made at two of the mission's outlying schools, one at Chikore, some 20 miles in a north-westerly direction from Silinda, and the second at Gogoyo, situated in the lowlands about 40 miles east in the territory of the Compagnie de Mozambique (Portuguese East Africa).

Practically all of our life history-studies were made at Mount Silinda. On leaving there, it was planned to make further observations which would give more information on the distribution and epidemiology of the infection and perhaps also allow of the continuation of life-cycle investigations in other districts where facilities were available for the examination of the natives. To this end, a survey of the intestinal fauna of natives was made in Livingstone, Northern Rhodesia, where Dr. E. F. Wallace, acting P.M.O. of the colony, kindly placed the facilities of the laboratory and native wards of the government hospital at our disposal. Subsequently, thanks to the enlightened interest of Doctor Feirera dos Santos, Chief of the Colonial Medical Service in Portuguese East Africa, and the wholehearted co-operation of Dr. M. M. Prates and members of his staff at the central laboratory adjoining the Hospital Miguel Bombarda, work was prosecuted for nearly a month in Lourenço Marques.

Space will not permit me to mention individually the many people who facilitated the work and to whom I am deeply indebted.

Suffice it to say that without the sympathetic co-operation of hospital and public health officials which was received at all points visited, the number of examinations made and the number of anthelminthic treatments given could not have been as large as they were.

I. THE EGGS OF *TERNIDENS DEMINUTUS* AND THEIR DIFFERENTIATION FROM THE EGGS OF OTHER STRONGYLES THAT MAY OCCUR IN HUMAN FAECES

The eggs of Ternidens are thin shelled and in shape are regularly ellipsoidal with broadly rounded poles. It is not, however, uncommon to find eggs which present a distinctly asymmetric outline, one side having a lesser convex curvature than the other. Such eggs strongly resemble the eggs of certain species of trichostrongyles and might be diagnosed as such, except that similar asymmetric eggs can be teased out of the gravid uterus of a female Ternidens under the microscope. Nevertheless, asymmetrical eggs are not to be regarded as typical, for they are easily outnumbered in the faeces by the symmetrical eggs upon which the diagnosis of Ternideniasis would usually be made. Somewhat comparably asymmetric eggs of the human hookworms are also encountered in the faeces.

In the faeces of ten human cases of Ternidens infection from whom the worms were later recovered, the mean dimensions of more than 100 eggs was found to be 84·07μ by 51·21μ. The size in this series of eggs ranges from 69·9μ by 54·4μ to 89·7μ by 52·4μ. The extreme range in length is from 69·9μ to 93·9μ, and in breadth from 46·8μ to 54·6μ. Proof that eggs bearing these measurements are actually those of Ternidens was secured from comparison with the fully developed eggs ready to emerge from the gravid uteri of worms of this species recovered after treatment of affected persons. In the freshly passed faeces, eggs of Ternidens are sometimes found not further developed than the first cleavage stage. It is not uncommon to find them containing four metameres but usually they are advanced to the third cleavage when they present eight cells. In fresh faeces they are not often advanced to the many-celled morula stage. Typical eggs of Ternidens in the early cleavage stage are shown in Plate VI, fig. 1.

It might easily be imagined from this description that there would be considerable difficulty in distinguishing between the eggs of Ternidens and those of the human hookworms. It is certain that the two have been frequently confused in the past. For their proper differentiation it might be necessary to take recourse to the calibrated ocular micrometer wherewith several eggs should be carefully measured before the diagnosis is made. However, when the technician is well acquainted with the magnifying power of his microscope, the fidelity with which a distinction may be drawn between two objects differing only a few micra in size without measuring is truly astonishing. Thus we were always able to tell from a simple inspection of the eggs whether a patient harboured Ternidens or hookworms or a double infection with both parasites.

The size of hookworm eggs as given by various writers differs so appreciably, especially in the higher limits, that the thought of questioning the accuracy of the measurements may arise. It has been suggested that the differentiation between Ancylostoma and Necator may be made on the basis of the size of the egg, but measurements show an overlapping in the range which vitiates the possibility of this distinction. However, it seems established as a fact that the eggs of Necator range slightly larger than those of Ancylostoma and consequently we need here only be concerned with the differentiation of the Ternidens eggs from those of Necator.

Stiles (1903), in his classical report on *Necator americanus*, states (p. 21) that ' the eggs of this species are from 64μ to 76μ by 36μ to 40μ.' These identical figures are repeated in several text books in English. Brumpt (1922) gives the mean size of Necator eggs as 70μ by 40μ, while Chandler (1929) states (p. 91) that ' the eggs of Necator range from 50μ to 80μ, but usually measure 70μ, and from 36μ to 42μ in breadth, averaging about 38μ to 40μ.' In Africa we made a large series of measurements of the eggs of *Necator americanus* in the stools of infested natives as well as fully developed eggs taken from the uteri of fresh worms. The mean size from the series was calculated to be 62·55μ by 40·8μ. The length varied from 58·2μ to 68·4μ and the range in breadth was from 38·8μ to 46·8μ.

Comparing these measurements with those of Ternidens eggs, a very appreciable difference is apparent. Even though the length of the largest Necator egg may closely approach the length of the

smallest Ternidens egg, the latter can always be distinguished by its greater breadth. The difference in the size of eggs of these two parasites is brought out much more strikingly if, instead of comparing length and breadth, one compares the volume of the egg, an index of which is secured by multiplying the length by the breadth squared. The figure arrived at in this manner for the average Ternidens egg is 209,368 ($=82{\cdot}77 \times 50{\cdot}3^2$) compared with 104,299 ($=62{\cdot}5 \times 40{\cdot}8^2$) for the average Necator egg according to our measurements. For the range of Ternidens eggs, the figures 196,844 ($=69{\cdot}9 \times 54{\cdot}4^2$) to 244,854 ($=89{\cdot}2 \times 52{\cdot}4^2$) are secured in contrast with a range of from 90,317 ($=57{\cdot}6 \times 39{\cdot}6^2$) to 128,122 ($=66{\cdot}8 \times 43{\cdot}8^2$) for Necator eggs. When the eggs of Ternidens are compared with those of Necator, side by side under the microscope, the difference is a very obvious one (see Plate VI, fig. 2).

That the difference in size between the eggs of Ternidens and the hookworm is sufficiently characteristic to make a differential diagnosis possible was proved to our own satisfaction on numerous occasions in Rhodesia. The confirmation of the diagnosis was secured by the passage of the expected parasites after treatments in cases where the infections involved Ternidens only, Necator only and mixed infections with the two parasites.

For the sake of completeness it seems desirable here to consider the question of the differential diagnosis by examination of the eggs of other strongyle infections that may affect man. Two, or possibly three species of nodular worms, namely *Oesophagostomum apiostomum* (synonymous according to Leiper with *O. brumpti*) and *O. stephanostomum* var. *thomasi*, have been found parasitising man in Central Africa and Brazil. The infection in man appears to be rare (three cases reported) and fortunately no cases of human Oesophagostomiasis appeared in our work in Rhodesia to necessitate further differentiation in the diagnosis of the eggs. On the other hand, the rate of infection of baboons, and two species of Cercopithecus with *Oesophagostomum apiostomum* and of sheep and pigs with other species of the genus was very high in this region and afforded an opportunity for the examination and measurement of its eggs. In the two human cases of Oesophagostomiasis that have been reported, the parasites were all immature and so dimensions of the eggs have not been given. Faust (1929, p. 353) states that ' the eggs of *O. brumpti*

closely resemble those of the hookworm and measure 60μ to 63μ by 27μ to 40μ. According to Walker (1910), the eggs of *O. apiostomum* in Philippine monkeys measure 73μ to 84μ by 44μ to 57μ and are passed in the morula stage. In the faeces of baboons infected with *O. apiostomum* the largest egg found by us measured 81·9μ by 39μ, the average length of many eggs was under 74μ and the average breadth 38·1μ. From these figures it would appear that the eggs of *O. apiostomum* could be distinguished from those of Ternidens by their uniformly smaller breadth.

Other parasites that may be encountered in man and whose eggs might have to be differentiated from Ternidens are the various genera and species of trichostrongyles. The eggs of *Trichostrongylus orientalis*, a common human parasite in Japan, measure 84μ to 90μ by 46μ to 50μ. According to Ransom (1911), the eggs of *T. colubriformis* (=*T. instabilis*) measure 73μ to 80μ by 40μ to 43μ, those of *T. probolurus* measure 76μ to 80μ by 43μ to 46μ, and those of *T. vitrinus* measure 84μ to 90μ by 46μ to 50μ. Another parasite of sheep that has been recorded from man is *Haemonchus contortus* whose eggs measure 75μ to 95μ by 40μ to 50μ.*

Specific diagnosis in human infections with these trichostrongyles is a matter of great difficulty for the relatively small worms inhabiting the mucosa of the stomach and small intestines usually occur in small numbers and are not readily secured for examination by the anthelminthic drugs employed for human treatment.

Where it is not possible to obtain the parent worms for identification, an indication of the parasites' taxonomic affinities, sometimes of their specific identity, may be obtained from a study of the third or infective stage larvae secured from the eggs by culture of the faeces. In the case of the trichostrongyles with its multiplicity of genera and species, the larvae often exhibit such homogeneous morphology that in our present state of knowledge even generic identification of the larva is not possible. The morphology of the infective larvae of the hookworms have been sufficiently studied and are characteristic enough to permit fairly easy recognition. However, when we come to members of the Oesophagostominae,

*The measurements given above usually have reference to eggs stored in the uteri of preserved material. In our experience the size of such eggs is usually smaller than the normal eggs passed in the faeces.

among which Ternidens is numbered, the larval forms, although quite distinguishable from the infective larvae of other human helminths, have never been made the subject of such meticulous morphological studies as to be specifically identifiable.

A brief account of the free living larval stages of Ternidens follows.

II. THE DEVELOPMENT AND MORPHOLOGY OF THE TERNIDENS LARVAE WITH SOME OBSERVATIONS ON THEIR BIOLOGY

Lacking a constant temperature incubator, all observations on the free-living phases of the life-history were perforce made at the temperature of the open air verandah where our work was done. The mid-day temperature at times would rise to slightly above 30° C., but usually in the rainy weather the days were distinctly cool and the night temperature as low as 13° C. These temperature conditions approximate those of the winter much more nearly than they do the summer heat.

The developmental history was observed on fertile eggs isolated from faeces or collected by teasing out the uteri of worms passed after anthelminthic treatment and brought into fresh water in a Syracuse watch glass or the well of a hollow-ground slide. In this way it was possible to follow the development of the embryo and its eventual hatching from the egg. In this artificial environment, the larva does not emerge before the lapse of 40 to 48 hours. The newly hatched larva presents the typical 'rhabditiform' morphology. It is from 0·30 mm. to 0·36 mm. in length and approximately 0·020 mm. in breadth at its widest point. The cylindrical cuticularised buccal cavity is 10·5μ long and 1·5μ broad. The oesophagus measures about 95μ in length and the genital primordium may be resolved as a refractile spindle-shaped body 11·2μ long. The extremity is drawn out into a flagella-like tail nearly 70μ long. The absence of nutriment from the medium leads to a rapid death of the larva but should a little faeces be added to the watch glass culture, the small rhabditiform larvae feed and grow, and in about 24 hours sheds its cuticle, passing into the second rhabditiform stage. This larva feeds actively and grows considerably. We have found such a rhabditiform larva to measure 0·62 mm. in length and 0·35 mm. in greatest width with the oesophagus 0·140 mm. long.

Under the relatively low temperature conditions that obtained, cultures made from a mixture of egg-laden faeces and sterilised soil or animal carbon did not commence to yield the third stage infective larvae until from eight to ten days. By analogy with the corresponding larval stages of other strongyle nematodes, the third stage larva of Ternidens must represent the infectious stage of the life cycle. This larva (Plate VI, fig. 1) ranges from 0·63 mm. to 0·73 mm. in length and from 0·029 mm. to 0·035 mm. in greatest breadth. It is enclosed in the sheath of the undiscarded second stage larval cuticle which it more or less completely fills. The cuticle of the larva is much more distinctly striated than is its enclosing sheath. Six minute punctiform papillae are to be observed under high magnification on the rounded head. A fine chitinised linear structure, representing either the rudimentary vestige of the second stage larval buccal tube or the incipient primordium of the fourth stage larva, may be detected leading to the lumen of the oesophagus. The organs of the head region appear to be very similar to those of the corresponding larvae of Oesophagostomes. In carmine-stained killed larvae examined under the magnification of a good oil immersion lens a pair of amphids or lateral line organs can be resolved. Each amphid consists of a more or less pyriform sac opening by means of a relatively long fine duct on the lateral aspect of the head. (See Plate VI, fig. 2.)

Owing to the presence of numerous subcuticular cellular elements and granular 'salivary' or oesophageal glands which obscures the view of the underlying structures, the determination of the finer details of the posterior part of the oesophagus is rendered rather difficult. The circum-oesophageal nerve commissure is situated about 0·1 mm. from the head end, slightly anterior to the terminus of the excretory tubule. The oesophagus is almost uniform in width throughout its length, with a slight bulbous enlargement at its posterior extremity. The oesophagus measures 0·15 mm. to 0·165 mm. in total length, being nearly one-quarter of the length of the body. Following on the end of the oesophagus are a pair of rather elongate sphincter cells which, in living specimens, are distinguishable from the cells of the true chyle intestine by their finer granulation. These sphincter cells are much contracted in fixed material. The intestine is composed of ten pairs of large

cells which alternate with each other on the dorsal and ventral walls. It opens through a narrower rectal tube to the anus, which is situated from 0·120 mm. to 0·145 mm. from the extremity of the body. The tail gradually narrows to a very fine point. The genital primordium measuring about 0·015 mm. in length is to be found hugging the wall of the intestine near its middle point.

No further development beyond the stage described above occurs in the free-living environment.

Biological Notes on the Third-Stage Larva.

The power possessed by third-stage Ternidens larvae of resisting the unfavourable effects of desiccation is midway between that displayed by such closely related genera as Oesophagostomum and Triodontophorus (which can withstand repeated drying for several days) and that displayed by the hookworms which are killed after a very short time in this condition. Ternidens larvae collapse and shrivel in a more or less distended position when the water surrounding them on a slide or watch glass evaporates in an atmosphere of low humidity. The internal structure becomes almost unrecognisable and large vacuoles appear under the cuticle, in the region of the intestine. Such larvae, however, slowly revive when water is added even after three days of drying, but this period cannot apparently be prolonged much beyond three days. As compared with trichostrongyle larvae, the larvae of Ternidens do not tend to roll up in a coil on becoming dry. When, in the process of reviving, water is added to the desiccated larvae, the enclosing sheath, which has become shrivelled, is often lost. Such denuded larvae appear to have lost their ability to withstand desiccation as tested on the glass slide. From these observations it is apparent that in nature the larvae of Ternidens are well equipped to withstand the frequently protracted periods of drought that is characteristic of their geographic environment.

Coupled with, and possibly enhancing, the desiccation resisting capacity is found that migrating instinct encountered in most Strongyle larvae for leaving the faecal mass in which they have been reared and to crawl up vertical surfaces such as the sides of a glass faecal culture vessel.

The movement of third-stage larvae in water in a watch glass is very lethargic compared with the rapid undulating movements of the corresponding stages of the hookworms. Although the rapidity of movement increases somewhat with an elevation of temperature, the larva does not show any marked response to the application of moderate heat. This absence of positive thermotropism makes the isolation of larvae through the Baermaun apparatus a tedious and inefficient process. Larvae continue to be discharged from faecal cultures placed in this isolation apparatus for a period as long as eight days. Although the reverse may not be true, by virtue of the absence of the phenomenon of thermotropism which is so strongly exhibited by the third-stage larvae of the Strongyloides, hookworms and other strongyles which can invade the host by the active penetration of the intact skin, it can be inferred that infection of the host with Ternidens occurs through the passive ingestion of the larvae with food or drink.

This inference can best be put to the test by Goodey's method of placing larvae on a ring of sheet cork covered with the fresh skin of a young mouse and floated in a vessel containing physiological saline solution at body temperature. If the larvae are capable of penetration they may be observed boring into the skin where they may later be demonstrated in serial sections. Unfortunately we were not able to carry out this experiment under favourable circumstances, and we consequently resorted to the otherwise questionable procedure of directly testing the infectivity of the larvae on the black skin of an African native and the white skin of a European. A small area of skin on the back of the forearm was shaved and washed and then laid on the stage of the binocular dissecting microscope for observation. In each case twelve healthy third-stage larvae in a few drops of water were placed on the skin. By careful focussing and arrangement of light, the larvae could just be discerned on the black skin but they exhibited no activity. No prickly sensation such as usually attends the penetration of hookworm larvae in the human skin were noticed. About twenty minutes after the water in which the larvae on the skin was originally contained had evaporated, a small quantity of water was again added and after a short time was carefully taken up in a pipette and transferred to a clean watch glass. In this manner eleven of the twelve larvae on the arm were

recovered in one experiment and nine of the twelve in the other. Most of these larvae on recovery were lying outstretched and motionless. Later they recommenced their usual languid movement. The individual from whose arm only nine of the twelve larvae were recovered has not shown signs of infection on examination up to eight months since the experiment was performed.

All the reactions of third-stage larvae mentioned above, namely, their sluggish movement, their tendency to migrate from cultures and to crawl up vertical surfaces, as for instance, of blades of grass, and their resistance to drying are indicative of the entrance into the host to produce infection being effected in a passive manner, i.e., by introduction of contaminated food or water.

III. THE INCIDENCE OF TERNIDENS INFECTIONS

(*a*) *Results of faecal examinations of native labourers of the Rand Gold Mines.*

Although a few tribes, such as the Zulus from Natal, do not show disposition towards underground mine work and are not found in large numbers in the native labour force, labourers for the Rand gold mines are derived from all points in the Union of South Africa. The largest numbers are recruited from various districts in Portuguese East Africa and are known as ' East Coast boys.' A relatively recent law of the Union government provides that natives from the tropical zone north of latitude 22° S. may not be accepted for underground mine work. Although this restriction holds theoretically, it is well known that natives originating from considerable distances north of this line will travel and offer themselves at labour-recruiting posts in the south for mine work. Owing to other attractions nearer home, few natives from Southern Rhodesia are indentured for mine work.

The huge labour forces on the Rand mines may, however, be considered as representative of the native population from a very extensive area of the African sub-continent.

During a period of seven weeks spent in the laboratory of Dr. Fischer at the City Deep mine hospital, in Johannesburg, faecal examinations were made on a total of 503 natives. All faecal examinations in Johannesburg as well as at other stations mentioned in this report were made by the writer by the Willis-Molloy brine

concentration method. At first our attention was directed mainly to the examination of 'East-coast' natives, but later examinations were made on 'Union boys' as well. From the outset careful attention was given to the differentiation of all Strongyle eggs, for the culturing of stools revealed the presence of trichostrongyle, Necator, and Ancylostoma infections in addition to Ternidens. Two hundred and forty-eight of the 503 natives were infected with hookworms. The eggs of species of trichostrongyles occurred in twelve cases. A diagnosis of Ternidens infection was made in fifteen individuals, eleven of whom belonged to the Shangaan, Nyambaan and M'Chopi tribes hailing from Portuguese East Africa; the remaining four were Xosa or Pondo boys from the Transkei territory of the Eastern Cape Province or Basutos from the Eastern Transvaal.

The differential diagnosis of Ternidens infections by faecal examination had not been made previously at the Rand Mines, but Dr. Fischer, who has been engaged for some time on a special investigation of the hookworm situation in the mines, submitted to me for identification a female specimen of Ternidens which had been passed together with hookworms by an East Coast native after anthelminthic treatment. Because of exigencies of the mine service, treatments could not be given to all of the fifteen cases that were diagnosed by us as infected with Ternidens. Seven were treated and Ternidens was recovered from four. In addition, the parasite was found at post-mortem in one case where a positive diagnosis had previously been made.

(*b*) *Results of faecal examinations at Mount Silinda.*

Mount Silinda, in the Melsetter district of Southern Rhodesia, is situated on latitude 20° S. on the south-eastern border of the colony overlooking the territory of the Compagnie de Mozambique in Portuguese East Africa. It is the principal seat of activities of the American Board of Foreign Missions in Rhodesia, maintaining a large mission school and farm for natives and linked up with many smaller schools in the region.

A total of 190 examinations of natives, mainly adolescent students of the Ndau tribe, were made at the Mount Silinda Institute. These students are drawn from a wide area of South-Eastern Rhodesia, a few coming from as far north as the Zambesi. Included in this

list are some patients who came from farms at some distance to visit the dispensary at the mission. Of the 190 patients, whose stools (with but a few exceptions) were examined only once by the brine levitation method, thirty-eight were declared negative for either hookworm or Ternidens infection. In the stools of forty the ova of hookworm only were found; the faeces of forty-eight yielded the eggs of Ternidens only, while in the faeces of sixty-four, the diagnosis of a double infection with both hookworm and Ternidens was made. Trichostrongyle eggs were observed in only two cases. The incidence of Ternidens in natives as determined by this procedure was consequently a little over 50 per cent. The incidence of Ternidens infection was very nearly the same for both sexes, it being but slightly higher (54 per cent.) in the forty-eight girls numbered in the group.

In addition to the 190 natives, the faeces of fourteen white missionaries who had spent periods of from two to more than thirty years at Mount Silinda and of eight children, some of whom were born and reared at the station, were examined but all proved to be free from helminthic infection.

(*c*) *Results of faecal examinations at Chikore.*

Chikore, situated about 18 miles from Mount Silinda, is the largest of the mission branch-stations and here the stools of 100 native students and dispensary visiting patients were examined. Twenty-three of these individuals were declared negative. Twenty-five harboured both hookworms and Ternidens. In the stools of twelve individuals only hookworm eggs and in forty the eggs of Ternidens only were observed. Trichostrongyle infections were diagnosed in two cases. The general incidence of Ternidens in Chikore is, consequently, at least 65 per cent.; the incidence in females was a little lower than in males. In the stools of none of the six white missionaries stationed at Chikore was it possible to find any worm eggs but a very light Ternidens infection was diagnosed in the case of the eight-year old daughter of one of the missionaries.

(*d*) *Results of faecal examinations at Gogoyo.*

Gogoyo, situated about 40 miles from Mount Silinda in the territory of Mozambique (P.E.A.), was the second large out-station of

the mission where examinations were made. The subjects, 124 in number, were largely school children, somewhat younger as a group than those of the other stations, and a few adult natives. The results of the survey show 19 (15·3 per cent.) negative, 71 (57 per cent.) infected with hookworm only, 1 (0·8 per cent.) infected only with Ternidens, and 33 (27 per cent.) infected with both hookworm and Ternidens. From these figures it is seen that at Gogoyo the infection rate is a little more than 27 per cent., while the incidence of hookworm infection amounts to nearly 84 per cent.

(*e*) *Results of faecal examinations at Livingstone.*

In the short period available for work in Northern Rhodesia it was possible to make an examination on only fifty-four individuals, patients in the Livingstone general hospital or labour recruits calling at the laboratory of vaccination The subjects were mainly of the Barotse tribe living in the Zambesi catchment area, but included also in this group are a number of natives from Angola and Nyasaland. A few of the natives had had hookworm treatment previously but the remainder had come directly from their kraals. Not a single case of Ternidens infection was encountered, but thirty-two of the fifty-four patients harboured hookworms. The absence of Ternidens was confirmed by culturing the stools containing the more suspicious eggs ; furthermore, treatment failed to dislodge any parasites but *Necator americanus*.

(*f*) *Results of faecal examination at Lourenco Marques and other East Coast ports.*

The stools of 323 natives were examined. Of these, 133 were hospital patients drawn mainly from the local population but many patients must also have come from far distant points in the colony. The stools of twenty-one native prisoners and of twenty-five labourers in the native compound derived largely from the southerly district of Inhambane were examined as well as stools from 123 askaris who, for service in the South, are recruited almost exclusively from tribes in the northern part of the colony. The distribution of this group, though small, is thus seen to be fairly representative of the entire colony. Seventy-four (45 per cent.) of the 163 native hospital patients, 9 (43 per cent.) of the 21 prisoners, 5 (20 per cent.) of

the 25 town compound labourers and 76 (62 per cent.) of the 123 askaris were found infected with hookworms (*Necator americanus*). Of the 323 donors of faecal specimens, only one was found infected with Ternidens. This infection in an askari was discovered in one of the last ten to be examined, and too late to allow us to treat the man for confirmation of the diagnosis. An interview with the man, who was called for questioning regarding his former habitat, revealed that he was in his first year of army service, previous to which he had spent many years in Southern Rhodesia. For a considerable part of this time he had actually lived in the immediate neighbourhood of Mount Silinda and Chikore, where our previous studies had shown Ternidens infections to be so prevalent.

In an endeavour to secure further information on the peculiar discontinuous distribution of Ternidens, the brief calls made by our steamer on the return voyage up the East Coast were utilised to make enquiries at the local hospitals. Thanks to Dr. Luiz de Almeida, it was possible to examine the stools of twelve natives in the government hospital at the port of Mozambique. Seven of these stools showed massive hookworm infections but no Ternidens eggs were seen. At the laboratory of the government hospital in Dar-es-Salaam, Dr. H. Burke Gaffney informed the writer that though faeces of patients were examined routinely, infections with Ternidens had not up to that time been made the subject of differential diagnosis but promised to direct an enquiry into the subject. Similarly, at the hospital in Tanga, enquiries showed that Ternidens infections had not been observed.

(*g*) *The examination of monkeys for Ternidens.**

The fact that in previous records of Ternidens infections, more have been reported from primates than from man, would naturally lead to the inference that the parasite finds more normal accommodation in simians than it does in man. A statement to this effect has recently been made by Cameron (1929).

Leiper (1908) reported the finding of a single specimen of *Ternidens deminutus* in a gorilla which died in London. Brumpt reports it

*In matters referring to the taxonomy of primates, I wish to record my obligation to Dr. Glover Allen, Curator of Mammals in the Museum of Comparative Zoology, whose authority I have followed.

from *Macaca sinica*, while Houdemer (1917) records that it has been found once in *Macaca* (=*Gynomolgus*) *fascicularis* in Tonkin. A report by Smith, Fox and White (1908), of the finding of *Globocephalus macaci* in a *Macaca nemestrinus* which died in the Philadelphia Zoo, probably also refers to the same species. The finding of Ternidens infections in the various African monkeys from the Rhodesian region was, consequently, expected and an effort was made at Mount Silinda and Chikore to secure specimens alive for infection experiments or dead for an examination of their natural parasites. At Mount Silinda, the most prevalent monkey is the blue monkey, *Cercopithecus leucampyx nyasae*, which occurs in the branches of the tall forest trees in large clans and subsists mainly on wild fruits. It does not appear to stray far from the forests. At Chikore, Gogoyo and elsewhere in this region, the common grey monkey, *Cercopithecus pygerethrus*, is occasionally seen. It does not seem to be in the habit of frequenting human habitation. Throughout this region the South African Chacma baboon, *Papio porcarius*, maraud the farms in large troops visiting the mealie lands and orchards, and wreaking great destruction to the crops.

Unexpected difficulties arose in our programme of procuring monkeys for the natives, who brought in all sorts of other animals for sale, were unable to secure living monkeys. However, we were able to shoot four specimens of *C. leucampyx* at Mount Silinda, a single *C. pygerethrus* was killed and brought in to us at Gogoyo, and as a result of a baboon hunt organised at Chikore, we secured six specimens of *Papio porcarius* of various ages. All of these animals were carefully examined for Ternidens, but all proved free of this parasite. All of the baboons as well as the specimen of *C. pygerethrus* harboured massive infections with the nodular worm, *Oesophagostomum apiostomum*. Whether the freedom from Ternidens infection of the species of Cercopithecus was due to their habit of avoiding human habitation and spending their lives in trees where contamination with the larvae of nematodes that develop in human excrement is unlikely, cannot, of course, be asserted but, in the writer's opinion, the absence of the infection in six baboons who live in much closer association with mankind in this region of high Ternidens incidence seems to be of greater significance.

An opportunity was fortunately provided for determining more

directly whether the baboon can act as a suitable host for Ternidens. A baboon aged about four months, and captured soon after birth, was used in an attempt at experimental infection. At the outset the animal was found to harbour massive infections with *Oesophagostomum apiostomum* and a species of Strongyloides, probably *S. fülleborni*, but the eggs of both of these parasites were easily distinguishable from those of Ternidens as found in human faeces. During a period of forty-two days, the baboon was fed on nine occasions with bread or water containing, in total, 3,650 third-stage larvae of Ternidens cultured from eggs taken from the uterus of the adult female worm or from the faeces of two of our most heavily infested cases. On no occasion was the animal seen to vomit its infective meal. The administration of the last 1,400 larvae was made in two doses respectively, eleven and eight days before the animal was sacrificed. Meticulous examination failed to reveal the presence of any Ternidens adult worms or developing forms.

It is felt that this experiment and the other observations detailed above are sufficiently adequate to justify the conclusion that in the region of Mount Silinda, at least, monkeys do not serve as a reservoir for Ternidens.

IV. THE INTENSITY OF TERNIDENS INFECTIONS IN RHODESIA AS DETERMINED BY TREATMENTS

Aside from an unusual hypersensitiveness that particular persons may exhibit to the presence of even a small number of helminths in the intestine, probably the chief factor that determines the pathogenic effect on the host is the number of worms that is harboured—in other words, the intensity of infestation. Other things being equal, the amount of harm suffered is more or less directly proportional to the number of worms occasioning the damage.

In the human host the only direct method of measuring the intensity of an intestinal helminth infection requires the use of an effective vermifuge that will expel the parasites from the intestine so that they may be collected and counted. The procedure is naturally unpleasant in its performance, tedious in the time required for the task and exceedingly difficult to carry out unless it is supported by co-operative spirit on the part of the patient.

Even with the hookworm, *Necator americanus*, which lives in the small intestine where it is readily affected by the oral administration of such a drug as carbon tetrachloride that has a high efficacy and, usually, a low toxicity, the difficulties of securing an accurate count of the worm burden in the field can only be overcome where the patients are prisoners and can be fully controlled so that the total amount of stool passed for a period of three days or more after treatment may be collected for sieving. Our attempt to determine the intensities of Ternidens infections in Rhodesia was confronted with even greater difficulties. In the first place, the location of the parasite in the large intestine where, theoretically at any rate, many of the worms may be protected by a cyst makes it necessary to use a drug that while being potent in its anthelminthic action is at the same time insoluble enough not to be absorbed in the anterior part of the alimentary tract, and that can safely be given in quantities sufficiently large to arrive at the seat of action in adequate concentration. This is one of the greatest obstacles to the effective treatment of oesophagostomiasis in sheep and other herbivora. This obstacle is not so great in man because of the relatively shorter intestine, but it still represents a grave handicap. In addition to the failure of the drug to dislodge all the worms we found it almost impossible to detain the patient for a sufficient length of time after treatment to make the collection of stools anything near as complete as we wished. The patients, even schoolboys who were under a certain amount of disciplinary control, were incapable of conceiving the possible value of data secured from the washing of stools which they held to be an unreasonable procedure, and would abscond when after delivering a few stools their patience was exhausted. Particularly did this apply in the case of the girls whose modesty as a rule would not allow them to remain around to deliver the material of the first few bowel movements after treatment. For this reason we could not expect that our treatments would yield much more than a control of the accuracy of our diagnoses.

However, in several instances we were able to have the co-operation of juvenile and adult boys to the extent of delivering their faeces for washing for a full two days after treatment. The results of these treatments in our opinion throw some light on the question of the grades of infestation.

The drugs that naturally proposed themselves to us for use in treatment were carbon tetrachloride, tetrachlorethylene and oil of chenopodium. The first of these had already been shown to possess a certain effectiveness for Ternidens in the hookworm treatments that had been given at Mount Silinda previous to our arrival. Carbon tetrachloride or tetrachlorethylene was given with water in quantities of from 3 to 5 c.c. according to the size of the patient with or without the admixture of 1 c.c. of oil of chenopodium. The medication was given on an empty stomach early in the morning, the patient having been instructed to take a very light meal the evening previous and to have an evacuation to decrease the quantity of bowel contents before treatment. The treatment was followed in an hour with a copious purge—usually one ounce of magnesium sulphate in solution. If, as in cases of chronic constipation, the purge had not the desired effect in a reasonably short time it was repeated. Occasionally a soap water enema was necessary.

A special, and usually successful, effort was always made to persuade those individuals who did not pass specimens of Ternidens in the stools of the first day after treatment to present the stools of the second day for washing. In this connection it is interesting to note that while examples of Ternidens are likely to appear in the bowel movements of the same day as treatment was received, the number of worms passed on the second day is usually considerably greater. The stools washed through the sieves on the third day as observed in the few cases that consented to remain for this period seldom yielded more than a very few worms; occasionally worms were absent on the third day and when present were never more than 10 per cent. of the numbers passed on the first two days. In most instances we were able to secure a sample of the faeces from patients from three to ten days after treatment enabling us, by the presence or absence of Ternidens eggs, to determine the gross effect of the treatment.

Carbon tetrachloride alone was given in the treatment of twenty-one individuals upon whose stools a diagnosis of Ternidens only or together with hookworm infection had been made. In nineteen of these cases the administration of the drug was followed by the passage of a greater or lesser number of Ternidens in the stools. In one of the two cases from whose stools specimens of Ternidens

were not recovered a later re-examination of the faeces showed the persistence of Ternidens eggs, while in the second case the patient, a woman, absconded on the day of treatment presenting no further opportunity for re-examination.

In six cases tetrachlorethylene only was administered in dosages varying from 4 to 5 c.c. In four of these cases specimens of Ternidens were passed. The absence of Ternidens egg at subsequent examination in only one out of three of these four cases showed that at least in two cases not all of the worms had succumbed to the treatment. In one of the two cases where 4 c.c. of tetrachlorethylene failed to expel any Ternidens worms, the patient was purged and as a second treatment 5 c.c. of the same drug mixed with 100 c.c. medicinal paraffin was administered in the form of a rectal enema. The enema was retained for about ten minutes, but since worms again failed to pass it is supposed that the enema did not reach high enough in the colon to come into contact with the parasite. The infection was shown still to be present when the patient's stool was later examined.

In a series of eight cases carbon tetrachloride or tetrachlorethylene in 3 to 4 c.c. doses was administered in combination with 1 c.c. oil of chenopodium. Specimens of Ternidens were passed in five of the eight cases removing all the worms in three cases so far as a single re-examination of the faeces could show. In two cases of this series where a number of worms were expelled and in the cases from whose stools after treatment no worms were retrieved, the eggs of Ternidens were found to persist. A second treatment of one of these refractory cases with a mixture of tetrachlorethylene and chenopodium had a similar negative result.

Even though the number of test treatments are, perhaps, too small to permit the drawing of satisfactory conclusions, and we have no means of knowing the proportion of the worms that were expelled from the host in the successful treatments, it will seem desirable to review the results of treatments for the sake of discovering the relative merits of the drugs used. Though producing the desired effect in the majority of cases, the use of either tetrachlorethylene or carbon tetrachloride in the quantities that we felt safe in administering in certain other cases fails to expel any worms. To attempt to use the number of worms expelled in each case where either

tetrachlorethylene or carbon tetrachloride was given as an index of the relative efficacy of these drugs would not yield reliable results since the number of worms left behind cannot be calculated from data now available. It may, however, be said that in a certain percentage of cases the use of either of these drugs appears to be effective in reducing the number of worms harboured to the point that eggs can no longer be found by a single application of the Willis-Molloy concentration procedure.

The combination of oil of chenopodium with either of these drugs does not, in our limited experience, produce a result superior to that obtained when either carbon tetrachloride or tetrachlorethylene is used alone. The failure of the drug to expel any Ternidens worms in a certain proportion of the cases may perhaps be adequately explained on the basis of its failure to arrive in the large intestine in concentration high enough to affect worms protected in cysts that are, in other cases, usually abandoned when the worms reach maturity.

In the treatment of oesophagostomiasis, where the parasites like Ternidens congregate in the large intestine where they are unamenable to attack by drugs administered *per os*, Veglia (1928) had promising results in the treatment of sheep with an enema containing powdered thymol in soap water and more recently Monnig (1929) has also advocated approach via the rectum after the sheep has been properly purged. Since it has been shown by Robbins (1929) that the absorption of carbon tetrachloride is low in the colon, the administration of high colonic irrigations containing rather large quantities of the relatively insoluble tetrachlorethylene in cases where the administration of this drug to man *per os* is without result, would appear safe and promising.

The Intensity of Infections

The numbers of worms that have been collected post-mortem from the few isolated cases of Ternidens infections previously recorded has been very small. These low grade infections, where less than ten worms have been collected from treated patients, were the rule at Mount Silinda and Gogoyo, but in many cases we are inclined to think that, had it been possible to secure and wash the stools for fully three days after the effective treatment of patients, the average

number of worms that would have been collected would have been appreciably more than the seventeen worms which we obtained as an average from fifteen cases from whom the worms passed were counted. This low count in all probability is also due to the fact that several of the cases included in our list had received a routine treatment for hookworm infection during the previous school session at Mount Silinda. Some of the heavier Ternidens infestations that were treated at Mount Silinda yielded from twenty-one to one hundred worms. At Chikore where no previous hookworm treatments had been given the intensity of Ternidens infestations as indicated by profusion of eggs in the Willis-Molloy faecal preparations was evidently higher. Here, twenty-three was the lowest number of worms recovered from any case that was detained sufficiently long for adequate worm counting after successful treatment; the maximum number was 103. At the City Deep Gold Mine the largest number of worms retrieved from a case (an East Coast Shangana) was sixty-seven.

V. PATHOLOGY

Because of our failure to find natural Ternidens infections in wild monkeys or to produce an infection experimentally in a young baboon, it becomes necessary to draw what conclusions we can on the pathology of the infection from the meagre observations made in a few human post-mortem examinations on very lightly infected subjects.

The City Deep native hospital in Johannesburg is provided with facilities for post-mortem examinations and about forty such routine examinations were attended during the period of our stay. The routine procedure in force at the time made it difficult to compare the number of the subject under examination and our records of faecal examinations so that we were unaware until after the necropsy that a positive faecal diagnosis of Ternidens infection had been previously made on two of the cadavers. This situation consequently afforded an undesigned objective test of the accuracy of the diagnosis made on the eggs of the parasite, but at the same time did not allow us to realise the full value of the examination as an opportunity for learning the detailed pathological picture of the infection.

The following notes were made on the special pathological findings in two cases that were involved with a Ternidens infection.

AUTOPSY No. 1. Subject a young adult East Coast native of the Nyambaan tribe. The cause of death was entered as lobar pneumonia with chronic pericarditis and acute nephritis as additional pathological findings.

Before the intestine was slit open, its contents were washed out by flushing with a stream of water. A diffuse enteritis of the small intestine was noted. The caecum showed nothing abnormal. In the transverse colon, five nodular cysts ranging from nearly three to a little less than five millimetres in diameter were found abutting onto the mucous surface internally and projecting prominently from the serous surface. In a fruitless endeavour to find larval worms, all of the cysts were dissected and were subsequently valueless for histopathological study. The cysts were calcareous in consistency, resembling those commonly found in the intestines of sheep parasitised with *Oesophagostomum columbianum*. A single female specimen of *Ternidens deminutus* was found attached to the wall of the descending colon.

AUTOPSY No. 2. Subject again a young adult of the Nyambaan tribe. The native mortuary assistant, whose duty it was to open the body and remove the viscera for examination, had, before my arrival, flushed the intestine with a stream of water and had, most unfortunately, already discarded the washings.

Examination of the large intestine showed seven dark patches about two to three millimetres in diameter with a paler area in the centre, on the peritoneal coat of the transverse colon. A similar patch was found on the proximal part of the caecum. From the internal aspect of the intestine, these lesions have the appearance of small circumscribed ulcerations, slightly elevated above the mucosa and with a small pore on the crater. No Ternidens worms were found in the intestine; they had, in all probability, been dislodged from the wall and thrown away with the faecal contents of the bowel.

In a third case in whose faeces the eggs of Ternidens had been found, death was due to septicaemia and permission could not be secured for post-mortem examination on account of the hazard.

After working in Johannesburg where Ternidens infections

proved to be uncommon and generally of low grade intensity, it was hoped that there would be greater opportunities for securing information on the pathology of the infection in places where the incidence was higher. Unfortunately, this expectation was not realised. On the veld, away from hospitals, opportunities for post-mortem examinations are rare, and when they do appear, must often be performed under conditions that are extremely unfavourable. The natives look with a superstitious suspicion on the practice of opening a cadaver, doubtless with ideas of witchcraft in their minds, and are quite opposed to the undertaking. It is only in those cases where there is a possibility of death by violence that the government in Southern Rhodesia requires that the district medical officer perform a necropsy. This requirement worked in our favour in procuring post-mortem examinations in two deaths of this order which occurred during our stay in the district of Mount Silinda.

The first was the death by suicide of a middle-aged male native. The body was cut down from a tree where it had been suspended for about forty hours in open country, some considerable distance from any supply of water. The examination was conducted on the ground, and so little water was available that it was not possible to wash out the contents of the intestine adequately to make a complete examination for helminths. The transverse colon was anchored to the peritoneum by old fibrous adhesions that indicated a former peritonitis in the lower abdomen. There were no signs of verminous cysts in the wall of the large intestine, but near the juncture of the ileo-caecal valve, the mucosa of the caecum showed a number of clusters of small papular elevations resembling those observed in the colon in the autopsies at Johannesburg referred to above. Although no worms were secured in this case, on account of the high incidence of Ternidens infestations in the district and the occurrence of the minute lesions in the mucosa of the colon, it seems probable that we were dealing with such a case.

The second post-mortem examination was on an aged male native who had probably had an epileptiform seizure while bathing and was drowned. My examination was focussed mainly on the large intestine which was again found adherent to the lower quadrant of the abdominal peritoneum. No cystic lesions were observed on the outer wall of the gut, but after the faecal contents had been

washed out the mucosa of the large intestine was found to be mildly injected in several localised areas and a few aggregations of elevated plaques with inflamed appearance were found on the mucosa of the colon. The contents of the caecum and colon when washed through a sieve yielded five specimens of Ternidens, characteristically flushed with bright blood in their intestines.

Summing up the fragmentary information gleaned from the four post-mortem examinations mentioned above, it becomes apparent that in the light infections that came under observation no acute or extensive pathology occurred. Ternidens normally inhabits the large intestine, but in only one case were large cystic nodules found.

The small papular elevations found in aggregations of three to five or singly on the mucosa of the caecum and colon in each case have not received any definite interpretation. Their occurrence in cases where the subject was known to be infected with Ternidens would seem to indicate a direct relationship. Occasionally the centre of these structures were red or blackened so as to suggest the seat of old ecchymoses. Unfortunately, these structures which could easily be distinguished from the macroscopic lymph follicles of this region of the intestine, lost any colour that they might have possessed when fixed in a formalin solution. They were exceedingly difficult to distinguish for imbedding and sectioning after our return to this country, and it was not possible to determine their origin. Because the worms when recovered either post-mortem or after anthelminthic treatment are always more or less engorged with blood, it is suggested that these small ulcer-like erosions indicate the site where they attach themselves to the wall of the gut to extract their pabulum. In analogy with what frequently takes place in sheep, pigs, etc., infected with species of the phylogenetically closely related Oesophagostomum, another possibility is that the lesions represent the remains of cystic cavities wherein larval development occurred. Justification for introducing this analogy, which, as it stands, does not appear germaine to the subject under discussion, seems to be desirable. The digression is all the more necessary if a note on the serious pathogenic potentialities of intense infection with Ternidens is to be sounded in this report. Under the prevailing conditions in that region in Southern Rhodesia where Ternidens infections are common, or in other parts of Africa and Asia where

it may be encountered, it will be necessary to wait, possibly for considerable time, until the opportunity for a post-mortem examination of an individual who has recently acquired an intense infestation with the parasite (such as was recorded in Thomas's Brazilian case of eosophagostomiasis) presents itself to a pathologist with special interest in the subject before a complete account of the pathology can become available.

The nematode family Strongylidae is constituted by a large number of genera and species which, in addition to their morphological similarity, have on broad general lines, so far as is known, comparable life-histories. Their eggs resemble each other, their larval development is similar, infection of the host is incurred by the ingestion of ensheathed third-stage larvae, and the great majority of the members after their final ecdysis live in the large intestine, especially the caecum and colon. Some produce verminous nodules in the intestine, while others, apparently, do not. Some attach themselves to the walls and suck blood ; others seem to live free in the lumen of the intestine, feeding on its contents (Ihle and Van Oordt 1923).

Many of these so-called ' palisade-worms ' are responsible for serious diseases in agricultural live stock ; yet the detailed histories of the wanderings of the larvae of various species after ingestion by the host before they establish themselves as adults in their definite habitat, are to a large extent unknown. In some the filariform larva is said to commence development by invading the wall of the small intestine (e.g., *O. apiostomum*) ; in other species the larva does not commence the invasion of the tissue until it arrives in the large intestine. Some attention has been devoted to the subject by such investigators as Ortlepp (1925) working with Triodontophorus and Thwaite (1928) working with *Strongylus equinus*, but it is the work of Veglia on eosophagostomiasis of sheep that furnishes us with the most complete account of the parasitic life of any member of this important family. In our present scheme of classification of the Strongylidae, Ternidens is included with the genus Oesophagostomum in the sub-family Oesophagostominae. Although morphologically its affinities appear to the writer to be just as close to the genus Triodontophorus, in which it was originally included as they are to the genus Oesophagostomum, the relationship

is close enough to warrant that the life-history of Ternidens within the host is broadly similar to that of the oesophagostomes. Furthermore, the facts that we have learned from our few post-mortem observations lend support to this view.

Abstracting from the works of Veglia (1924 and 1928), the salient features of the life-history and pathology of *O. columbianum* in sheep are briefly as follows :—

Third-stage larvae when administered in a drench to young lambs follow the stream of ingesta through the stomach and intestine so far as the lower part of the ileum and the large colon. Only when there is an unusual retention of larvae in the small intestine due to the various causes of obstruction and acute stasis, do the larvae tend to invade the mucosa of jejunum and ileum with the production of a catarrhal inflammation. Occasionally cysts containing larvae or in process of calcification are found in the walls of the ileum. Soon after arriving in the colon, the larvae commence to burrow into the depths of the mucosa. White granular looking cysts measuring from 0·2 mm. to 0·3 mm. in diameter with thin elastic walls are formed by the mucous membrane around the larvae. In these cysts the larvae become quiescent and prepare for the third metamorphosis. The large intestine appears red and oedematous, and soon naked eye examination of the mucosa of the colon shows numerous petechiae, some with a yellowish centre. This yellowing is due to narcotic complications within the cysts. By the seventh day after infection. the larvae have attained the fourth stage of development and are commencing actively to migrate from the cysts. About half the nodules contain one or more larvae ; the other half have emptied into the thick catarrhal coat of the mucosa. There is an ulcerative and suppurative colitis producing colic and mucous diarrhoea in heavy artificial infestations. The eighth day sees practically all the larvae abandoning the cysts for the lumen of the intestine. A number of small yellow centres are visible on the external intestinal wall, apparently produced by very deep necrotic ulcerations. From this time until the thirtieth day, the larvae which have migrated from the cysts remain in the lumen of the caecum, large colon and portion of the small colon for the remainder of their lives. They attain sexual maturity at the end of this time and eggs begin to appear in the faeces at the end of forty days.

It would appear that in lambs emergence from the cysts occurs

immediately after the completion of the third metamorphosis and that an encysted larva situated too deeply in the intestinal wall or too thickly encased by an adventitious fibrous covering may never emerge at all ; or at least that its migration may be indefinitely delayed. In several cases Veglia observed that larvae found their way into the peritoneal cavity after leaving the cyst. Their presence in the peritoneal cavity gave rise in several cases to acute septic fibrinous and purulent peritonitis, which usually proved fatal to the host. With the exit of the larva from the cysts, a small ulceration is produced, which, however, cicatrizes as a simple wound without noticeable suppuration. By the twelfth day after infection, many of the ulcerations may be well cicatrized and by the thirtieth day all macroscopic traces of the cicatrized ulcers may have completely disappeared. The caseous and calcareous nodules usually noted at the post-mortem of infected sheep are apparently incidental sequelae resulting from traumatic, necrotic and bacterial complications following original cystic ulceration.

In further investigations on oesophagostomiasis in sheep, Veglia (1928) devotes a chapter to the symptomatology of the infection, of which the following summary will, I think, prove of interest. The symptomatology of the infection is divided into three stages corresponding to phases of the parasite's life-cycle. In the first few days when there are few local lesions in the intestinal mucosa, the symptoms are slight. From the fifth to the seventh day there is a diarrhoea with faeces sometimes streaked with blood. In heavy infestations, death within the first fourteen days is due to a necrotic enterocolitis. Should the lambs survive this period of the infection they enter into a toxic phase of the disease which does not reach its acme for several months. There is an alternation of the mild diarrhoea and constipation. The visible mucous membranes are not particularly pale. Gradually the animals lose weight and there is abdominal tension. By the third or fourth month, some lambs show a pronounced muscular weakness of the hind quarters. A paresis sometimes follows and, although appetite and digestion is good, the animal gradually dies. At autopsy the few nodules found in the intestine are small and calcareous and adult worms are found in the colon. The internal organs are rather pale ; the liver, spleen and heart are particularly atrophic.

The spontaneous elimination of adult specimens of eosophagos-

tomes has been frequently observed particularly at times when the animal is afflicted with diarrhoea. To the writer's view, this elimination of the parasites in sheep represents the natural method whereby the mass of infestation becomes spontaneously reduced. The process is rendered mechanically simple by the fact that, as a rule, the adult oesophagostomes do not attach themselves to the mucosa but are found merely in its proximity where they apparently absorb their nutrition from the intestinal contents. By the time an animal is full grown, it has lost a large proportion of its oesophagostomes, and under natural conditions appears to be resistant to the acquisition of new worms in large numbers.

In domesticated animals at any rate it is evident that the serious effects of oesophagostomiasis come in the early migratory phases of the worm's life-cycle. This would also appear to be the case in man since the only two records of this infection (Brumpt's case in East Africa and Thomas' case in Amazonia) which were acute enough to be fatal involved worms that were encysted in the wall of the intestine and were without a single exception sexually immature. If the intestinal phases be similar in the life-cycle of Ternidens, it readily explains the paucity of worms that were found in adults and the absence of gross lesions that characterised our post-mortem findings.

The pathology of oesophagostomiasis in monkeys as described by Weinberg (1909) and in man as described by Thomas (1910) is in every way comparable to that in sheep. Weinberg, in addition, stresses the toxic complications of the infection, stating that the worms secrete haemotoxic substances analogous to those produced by certain strongyles in the horse whose life-histories have similar grave pathological aspects. It seems warranted, therefore, to infer that Ternidens is potentially as pathogenic to man as are its relatives in their respective hosts.

However, working as we were, only with the terminal stages of Ternidens infection, the exhibition of acute symptoms was scarcely to be expected, especially so since the majority of the cases dealt with, harboured a relatively small number of parasites. Even the cases with a burden of 70 to 100 worms were free from obvious signs of disease and in no case were any specific symptoms elicited. In view of the fact that Ternidens is an avid feeder on blood and is

considerably larger than the hookworms, it would be expected that it would be at least as provocative of an anaemia as is the notorious *Ancylostoma duodenale*, but not even in our most intense cases of Ternidens infestation was the haemoglobin as measured on the Talquist scale consistently any lower than it was in lightly—or non-infected cases. Furthermore, the prevalence of *Necator americanus* infestations and the occurrence of endemic malaria and bilharziasis complicate the local situation, and does not permit one to ascribe any syndrome to Ternidens unless other infections can be eliminated as contributory factors. Nor was it possible under the existing conditions to arrive at any conclusions on the subject of possible causes of low grade anaemias by following up cases in which treatment had effected a diminution or complete elimination of the Ternidens infestations. It is, however, reported that after a mass treatment of parasitised individuals at Mount Silinda, the students are noticeably more energetic and that dispensary treatments show a marked falling off in number.

SUMMARY

1. *Ternidens deminutus*, a nematode parasite previously recorded only twice from man, was found in 15 of 503 natives coming from various parts of South Africa and examined in Johannesburg. Its incidence was found to be from 50 per cent. to 65 per cent. among the natives in the south-east section of Southern Rhodesia. It was not observed in fifty-four natives of various tribes examined in Northern Rhodesia, and infection with this parasite appears to be equally rare among the natives of Portuguese East Africa examined in Lourenco Marques.

2. The parasite has been reported on various occasions in primates from Africa and Asia, but in the region of high human incidence in Rhodesia, it was not found in baboons nor in two species of Cercopithecus. An attempt to infect a young baboon by feeding with larvae derived from human cases failed.

3. The eggs bear a striking similarity to those of *Necator americanus*, but may be distinguished from these by their larger size.

4. The infective third-stage larva resemble those of species of Oesophagostomum and like the latter were found to be incapable of

penetrating the human skin ; infection of the host in all probability takes place by ingestion.

5. The adult parasite lives in the large intestine where in one post-mortem examination it was found in association with calcareous cysts in the wall of the colon. In analogy with its close relative Oesophagostomum, Ternidens appears to be capable of considerable pathological damage, especially in the earlier stages of its development.

6. As many as 103 specimens have been recovered from the stools of a patient after treatment with carbon tetrachloride or tetrachlorethylene *per os*, but in many cases this form of medication does not prove entirely effective. As a rule the number of worms recovered from patients after treatment has been low and such cases exhibited no evident symptoms of the infection.

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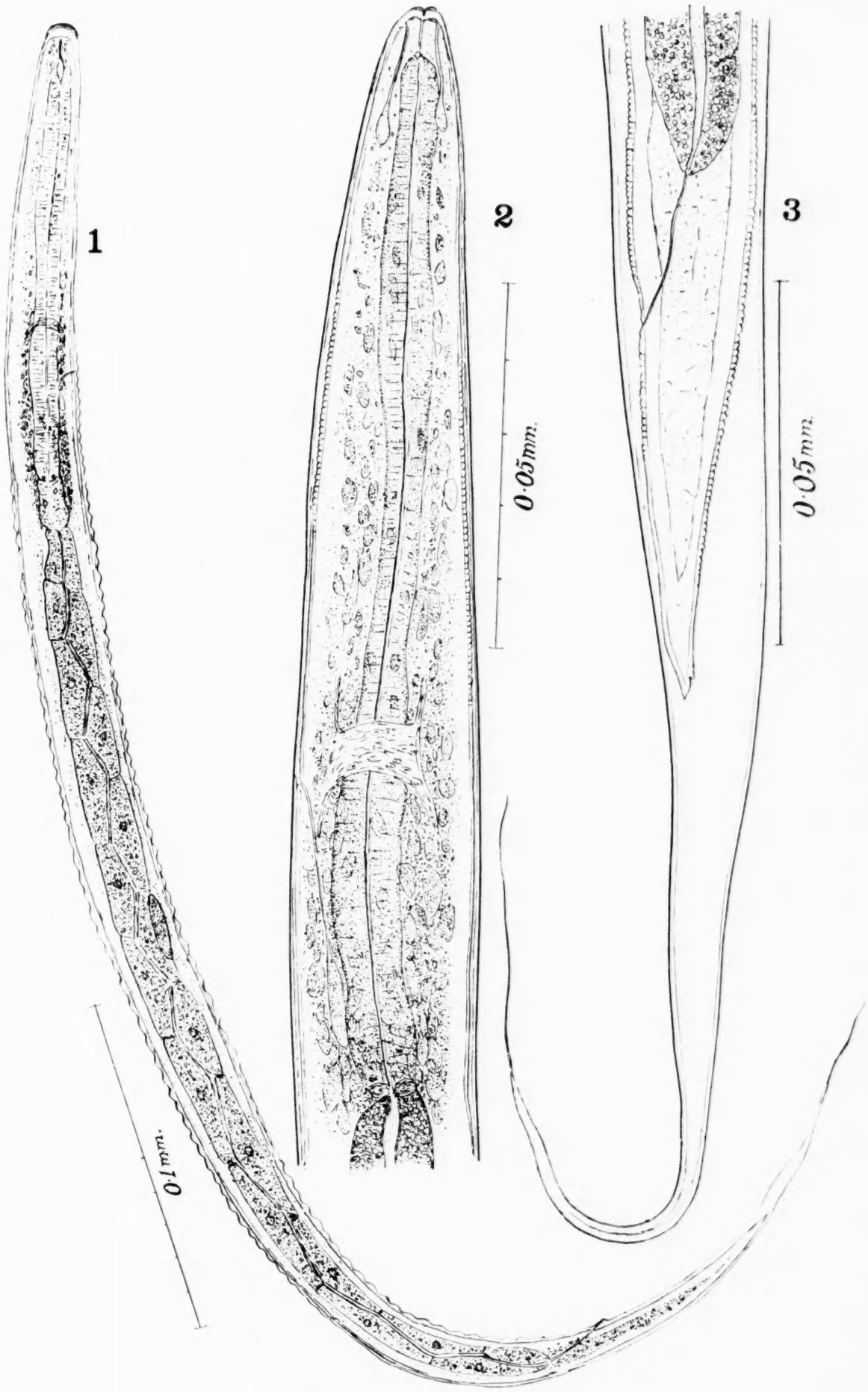
PLATE V

EXPLANATION OF PLATE V

Third-stage larva of *Ternidens deminutus*.

Figures 2 and 3 are drawn under high magnification to show details of structures in the anterior and posterior regions respectively, of the larva.

PLATE V



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EXPLANATION OF PLATE VI

Fig. 1. Eggs of *Ternidens deminutus*. × 560.

Fig. 2. Eggs of *Necator americanus* together with one egg of *Ternidens deminutus*.

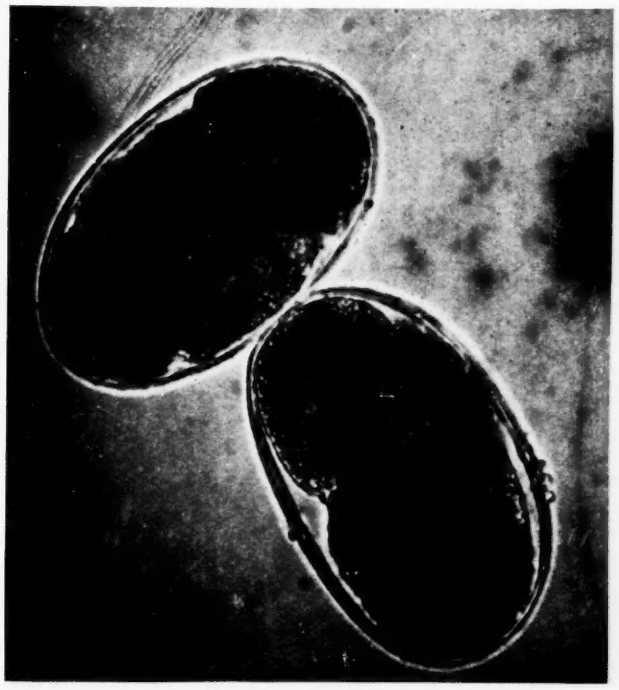

FIG. 1

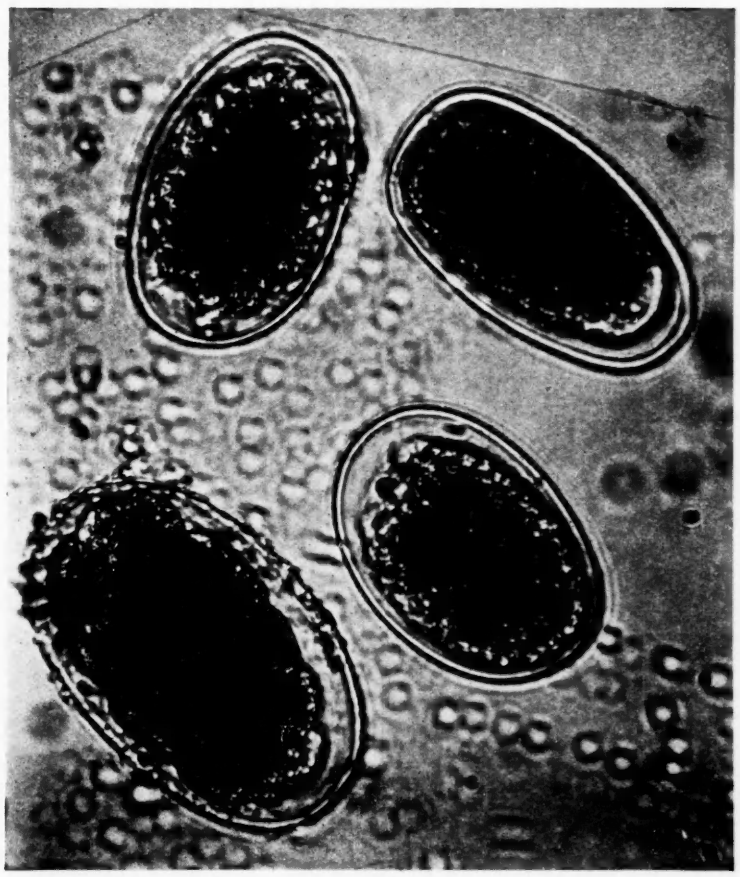

FIG. 2

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FEULGEN NUCLEAL REACTION AND THE MALARIAL NUCLEUS

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Our knowledge of the structure and chemistry of the nucleus of the protozoa is still very indefinite and incomplete. Biologists recognize that chromatin is present in varying degrees in nuclear structure but, as Wenyon (1926) says, 'it is an exceedingly difficult matter to give a precise definition of the term nucleus.' Feulgen and Rossenbeck (1924) and Feulgen (1926) have, however, made an important contribution to the study of the micro-chemistry of the nucleus which has received much recognition. Their conclusion may be briefly summarized as follows. In the composition of the nucleus there are two groups of nucleic acids, a pentose-containing group and a thymonucleic acid group. This thymonucleic acid group consists of four nitrogen-containing parts—guanin, adenin, thymin and cytosin—together with phosphoric acid molecules and carbohydrates. When guanin and adenin are subjected to a partial acid hydrolysis reducing groups are produced which in combination with sulphuric acid fuchsin produce in turn aldehydes yielding a vivid red colour characteristic of Schiff's aldehyde reaction. In contrast to the nucleus the cytoplasm of cells contains no such group capable of producing aldehydes and the reaction is therefore negative. This reaction shown by the development of a red coloration is known as Feulgen *Nucleal* reaction. Feulgen considers it to be a true micro-chemical test indicative of the presence of nucleo-protein in the form of thymo-nucleic acid.

The Feulgen nucleal reaction has recently been applied to the study of nuclear chromatin and other structures by several workers. M. Robertson (1927) (1928) made extensive use of it in the study of the cytology of *Trypanosoma raiae, Bodo caudatus* and

Heteromita with special reference to their parabasal body and mode of division and found it ' a most valuable addition to the staining methods at present in use.' Cowdry (1928) applied it to the study of the micro-chemistry of nuclear inclusions in certain virus diseases and found the reaction wholly negative with the inclusions of chicken pox, herpes, virus III disease of rabbit and salivary gland disease of guinea-pigs. Cowdry (1928) also applied it to the study of fibroblasts and sarcomatous cells in tissue cultures and found the latter gave a more intense Feulgen reaction.

It was therefore thought to be of some interest to apply the reaction to the study of the ' nucleus ' of the human malarial parasite. From cases showing numerous ring forms of *Pl. falciparum* and from cases with various stages of the sexual and asexual forms of *Pl. vivax* smears were made and fixed both by drying in the air and by sublimate alcohol (Romeis, 1928). The modification of the Feulgen nucleal reaction adopted by Robertson (1927) was employed. The acid hydrolysis was allowed to take place in five to six minutes and all the reagents were freshly prepared before use. By this method the nuclei of these forms of malarial parasites gave a wholly negative Feulgen reaction, whilst in the same preparations the nuclei of the leucocytes gave a distinctly positive reaction. This result was unexpected both in view of the great assistance obtained by Robertson with the reaction in her work mentioned above, and in view of Cowdry's statement that ' the material responsible for the Feulgen reaction which is probably thymonucleic acid, while not quite so ubiquitous, is nevertheless an essential constituent of animal cells including the pathogenic protozoa.'

SUMMARY

The modification of the Feulgen Nucleal reaction applied here indicates

1. That thymonucleic acid is absent in the composition of the nucleus of certain forms of human plasmodia.

2. That the nuclei of these forms of plasmodia differ chemically from the nuclei of the protozoa studied by Robertson, viz., *Trypanosoma raiae, Bodo caudatus* and *Heteromita.*

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OBSERVATIONS ON THE CEREBRO-SPINAL FLUID IN NINE CASES OF RHODESIAN SLEEPING SICKNESS DURING TREATMENT WITH BAYER 205 AND TRYPARSAMIDE

BY

J. F. CORSON

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These observations, made at Maswa in Tanganyika Territory, were undertaken in order to see what effect the trypanocidal drugs, Bayer 205 and Tryparsamide, might have on the cerebro-spinal fluid in Rhodesian sleeping sickness. It was thought possible that somewhat frequent observations, made during periods of from about six months to a year, on patients continuously under treatment, might yield some data that could be a guide to prognosis and treatment. The people of this district are by no means averse to medical treatment, the injection needle being much in demand for many ailments. They have been accustomed to the application of sleeping sickness measures since 1922, when the Mwanza outbreak was discovered here. During recent years the incidence of sleeping sickness in this district has greatly diminished, and the number of new cases that came for treatment during 1930, when these observations were made, was small. It can be seen from the serial numbers 272 to 276, the cases not having been selected, that, in spite of seven or eight years' experience of the high mortality of sleeping sickness, the African native of these parts does not usually trouble to come for treatment until the disease is somewhat advanced.

Much importance has naturally been attached to the occurrence of a reduction in the cell-count of the cerebro-spinal fluid during and following medicinal treatment, especially a reduction to near the normal figure and, still more, maintenance at this low figure for some months. The cell-count did not fall to normal in any of these cases but, in Cases 273, 274, 275, and 276, there was a considerable

Period of observation and treatment—Months ...			...	0·5	1	1·5	2	2·5	3	3·5	4	4·5
Case 222 Shaushi Relapse	Bayer 205, grammes ...		...	...	...	...	...	...	...	...	...	...
	Tryparsamide, grammes ...		...	...	...	...	9	...	12	...	...	16
	C.S.F.	Trypanosomes ...	...	...	+	...	0	...	+	...	...	+
		Cells, per c.mm. ...	...	...	680	...	300	...	200	...	...	460
Case 256 Masunga Lonsiku Relapse	Bayer 205, grammes ...		...	...	4	...	...	...	...	...	...	...
	Tryparsamide, grammes ...		...	...	...	...	15	...	12	...	...	16
	C.S.F.	Trypanosomes ...	...	...	+	...	0	...	+	...	...	+
		Cells, per c.mm. ...	...	...	710	...	310	...	110	...	...	800
Case 276 Mugunira	Bayer 205, grammes ...		...	...	4	...	...	...	...	...	...	...
	Tryparsamide, grammes ...		...	...	R	...	6	...	R	...	15	...
	C.S.F.	Trypanosomes ...	...	...	0	...	0	...	...	...	0	...
		Cells, per c.mm. ...	...	...	94	...	25	...	...	...	7	...
Case 275 Kimbuya	Bayer 205, grammes ...		...	...	5	...	...	...	...	...	...	...
	Tryparsamide, grammes ...		...	...	...	...	12	...	...	...	18	...
	C.S.F.	Trypanosomes ...	...	...	0	...	0	...	...	...	0	...
		Calls, per c.mm. ...	...	...	142	...	14	...	...	...	6	...
Case 274 Kilum Gumika	Bayer 205, grammes ...		...	...	2	...	...	3	...	...	...	...
	Tryparsamide, grammes ...		...	...	...	...	...	...	...	...	...	...
	C.S.F.	Trypanosomes ...	...	...	0	...	...	0*	...	...	...	...
		Cells, per c.mm. ...	...	...	100	...	...	305	...	...	...	...
Case 140 Nandi Relapse	Bayer 205, grammes ...		...	1	1	...	1	...	...	...	...	...
	Tryparsamide, grammes ...		...	...	...	...	13	...	...	21	...	...
	C.S.F.	Trypanosomes ...	0	0	0	...	0	...	.....	0	...	...
		Cells, per c.mm. ...	95	120	635	...	230	...	...	106	...	...
Case 273 Dindai	Bayer 205, grammes ...		...	...	1·5	...	...	1	...	1	...	...
	Tryparsamide, grammes ...		...	...	...	R	...	...	R	...	...	...
	C.S.F.	Trypanosomes ...	+	...	0	...	...	0*	...	0	...	...
		Cells, per c.mm. ...	520	...	215	...	...	485	...	450	...	...
Case 272 Masunga Lambo	Bayer 205, grammes ...		...	...	...	...	4	...	...	...	...	...
	Tryparsamide, grammes ...		...	...	...	...	...	...	6	...	...	...
	C.S.F.	Trypanosomes ...	...	...	...	...	0	...	0	...	...	...
		Cells, per c.mm. ...	...	...	...	...	1616	...	116	...	...	...
Case 255 Lutambi Relapse	Bayer 205, grammes ...		...	...	...	...	...	...	...	...	1	...
	Tryparsamide, grammes ...		...	...	11	...	9	...	...	...	4	...
	C.S.F.	Trypanosomes ...	0	...	0	...	0	...	...	...	0	...
		Cells, per c.mm. ...	420	...	70	...	60	...	...	...	212	...

* = rat infected.

R. = blood relapse.

·5	5	5·5	6	6·5	7	·5	8	8·5	9	9·5	10	10·5	11
…	…	…	…	…	…	…	…	…	…	…	…	4	…
16	…	10	…	…	14	…	…	10	…	…	…	9	…
+	…	0	…	…	+	…	…	+	…	…	…	0	…
60	…	1150	…	…	493	…	…	806	…	…	…	34	…
…	…	…	…	…	…	3	…	…	…	…	…	…	2
16	…	10	…	…	…	12	…	…	18	…	…	…	12
+	…	0	…	…	…	0*	…	…	0	0	…	…	0
00	…	257	…	…	…	188	…	…	239	287	…	…	37
…	…	…	…	…	…	…	…	…	…	…	…	…	…
…	…	…	21	…	…	…	R	…	…	…	…	…	…
…	…	…	0	…	…	…	…	…	…	…	…	…	…
…	…	…	10	…	…	…	…	…	…	…	…	…	…
…	…	…	…	…	…	…	…	…	…	…	…	…	…
…	…	…	12	…	…	…	12	…	…	…	…	…	…
…	…	…	0	…	…	…	0	…	…	…	…	…	…
…	…	…	5	…	…	…	37	…	…	…	…	…	…
…	…	…	…	…	…	…	…	…	…	…	…	…	…
…	…	36	…	…	…	18	…	…	…	12	…	…	…
…	…	0	…	…	…	0	…	…	…	0	…	…	…
…	…	24	…	…	…	17	…	…	…	16	…	…	…
…	…	…	…	…	…	…	…	…	…	…	…	…	…
…	15	…	…	…	9	…	…	…	…	…	…	…	…
…	0	…	…	…	0	…	…	…	…	…	…	…	…
…	62	…	…	…	384	…	…	…	…	…	…	…	…
…	…	…	…	…	…	…	…	…	…	…	…	…	…
…	10	…	…	…	18	…	…	…	14	…	…	…	…
…	0	…	…	…	0	…	…	…	0	…	…	…	…
…	45	…	…	…	24	…	…	…	18	…	…	…	…
…	…	…	…	…	…	…	…	…	…	…	…	…	…
…	18	…	…	…	18	…	…	…	10	…	…	…	…
…	0	…	…	…	0	…	…	…	+	…	…	…	…
…	?	…	…	…	713	…	…	…	1063	…	…	…	…
…	…	…	…	…	…	…	3	…	…	…	…	…	…
…	…	10	…	…	…	…	18	…	…	…	…	…	…
…	…	0*	…	…	…	…	0	…	…	…	…	…	…
…	…	580	…	…	…	…	49	…	…	…	…	…	…

reduction lasting for some months. In Case 276 a relapse, with fever and trypanosomes in the blood, occurred about the eighth month though there had been a reduced cell-count for several months before. Case 273 has remained weak, with wasted muscles, throughout the period. In Case 275 the number of cells rose to thirty-seven after having been as low as six and five during preceding months. The cell-count in Case 272 did not seem to be in keeping with his cheerful demeanour and apparently good bodily health. Lumbar puncture prevented, for the time being, an error of judgment as to his condition. A total of 50 grammes of tryparsamide in seven months had no apparent effect on the cerebro-spinal fluid. Case 255 has been previously reported on (Corson 1930a). During treatment with tryparsamide the trypanosomes frequently reappeared in the blood and infected rats. Since receiving Bayer 205, after three months, there has been no blood relapse. Cases 222 and 256 have also been the subject of a previous paper (Corson 1930b). The disease has progressed, more or less steadily, in both. At the time of the last lumbar puncture Case 256 was very weak and tremulous and had incontinence of urine. A few days later he developed a bed-sore on the hip. The reduction in the cell count did not indicate improvement. Case 222 has shown progressive mental impairment and the patient is now dull and stupid, negligent in personal habits and losing flesh.

In these cases the dose of Bayer 205 was almost invariably 1 gramme. That of tryparsamide ranged from 2 to 4 grammes, but was usually either 2 or 3 grammes, given at weekly intervals. The cell-counts were made with a Fuchs and Rosenthal slide. The details of the observations are set down in the accompanying table (pp. 192-193).

CONCLUSIONS

The significance of a reduction of the cell-count of the cerebro-spinal fluid to a normal figure cannot be judged from these cases. No inferences as to prognosis in general and treatment can be drawn from the observations. It would seem that the prognosis in all these cases is unfavourable, and that treatment should be continued for want of something better and to guard others.

The observations indicate that neither Bayer 205 nor tryparsamide can be regarded as likely to cure advanced cases of Rhodesian sleeping sickness, and this is in accordance with clinical experience.

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NEW ZEALAND *CERATOPOGONIDAE*

BY

A. INGRAM

AND

J. W. S. MACFIE

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CERATOPOGONIDAE are no doubt abundant in New Zealand, but hitherto they have been almost completely neglected, perhaps because the majority do not attack man. Up to the present, so far as we have been able to ascertain, only three species have been described, but many more certainly await description, and Tonnoir (1924), indeed, has stated that he knows of ' about a score of them.' The following notes on a small collection preserved in the British Museum (Natural History), London, may therefore be of interest, notwithstanding the fact that all the species described belong to genera with a wide or world-wide distribution, and possess characters that cannot be regarded as peculiar to the region. With the exception of Ohakune, all the localities in which the specimens were collected are in the South Island.

We have to thank Dr. F. W. Edwards for very kindly giving us the opportunity of examining the collection.

Forcipomyia parvicellula, n.sp.

A small brown species, with two large pale spots on the anterior border of the wing, and almost uniformly brown legs ; without definite scales.

FEMALE. Length of wing, 1·2 mm. ; greatest breadth of wing, 0·4 mm.

Head dark brown. Eyes bare. Palpi pale brown. Antennae pale brown : segments 4 to 14 forming a continuous series, without an abrupt change of shape between the tenth and eleventh segments, all oviform and about the same size, about 10 by 7 units* ; the last segment larger, about 14 by 8 units, without a stylet. The combined

* The unit used is approximately 3·6μ.

lengths of segments 11 to 15, 4 to 10, and 3 to 10 about 54, 70, and 80 units respectively.

Thorax dark brown, well clothed with hairs. Scutellum dark brown, bearing rather numerous bristles and hairs. Post-scutellum dark brown.

Wings densely clothed with macrotrichia, many of which are almost scale-like. Adorned with pale spots as follows:—on the anterior border two large spots, one situated just beyond the end of the costa, and the other near the tip of the wing; at the root of the wing a large pale area extending distally as far as the level of the cross-vein; and traces of less definite spots over the middle of M2 and in the fork of Cu. Costa reaching not quite to the middle of the wing. First radial cell obliterated; second very small. Fork of Cu at about the level of the base of the second radial cell. Halteres with white or cream-coloured knobs.

Legs almost uniformly brown; the knees, however, a little paler than the rest; without scales. Tarsal ratio (T.R.) about 1·3.

Abdomen dark brown, without scales. Spermathecae two, highly chitinised, oval, sub-equal, about 53μ by 39μ; the duct chitinised for only a short distance, about 5μ.

GOVERNOR'S BAY, 17.viii.1923, 2♀♀ (J. F. Tapley).

Forcipomyia tapleyi, n.sp.

A dark brown species of moderate size, with unadorned wings, very dark brown halteres, banded legs, and T.R. about 0·8; without scales.

FEMALE. Lengths of wing, 1·7 mm.; greatest breadth of wing, 0·6 mm.

Head dark brown. Eyes bare. Palpi darkish brown; the third segment longer than the fourth and fifth together, inflated at the base, and furnished with a large sensory pit; the lengths of the last three segments in one specimen 29, 14, and 11 units respectively. Antennae brown; segments 4 to 10 flask-shaped, in one specimen measuring from 15 by 9 to 17 by 8 units, armed with long pointed spines; segments 11 to 14 sub-equal, not flask-shaped but tapering distally a little, about three times as long as broad (20 to 22 by 6 or 7 units); the last segment rather longer (25 units) and broader,

terminating in a nipple-like process. The combined lengths of segments 11 to 15, 4 to 10, and 3 to 10 measuring in one specimen 112, 119, and 135 units respectively.

Thorax dark brown, well clothed with hairs. Scutellum dark brown, bearing numerous bristles and hairs. Post-scutellum dark brown.

Wings unadorned, without pale spots, but with the wing surface somewhat infuscated especially at the anterior border; densely clothed with dark macrotrichia but without scales. Venation as usual. Costa reaching to about the middle of the wing. First radial cell obliterated; second well formed, of the usual shape. Petiole of the median vein about as long as the cross-vein. Fork of Cu at about the level of the end of the costa. Halteres with very dark brown knobs.

Legs rather pale brown, with pale knees and rather indistinct darker bands as follows:—on the femora at the base and a little before the knees; and on the tibiae at the apex, and on the four anterior legs (but less distinctly on the middle legs) just beyond the knees. Legs well clothed with hairs, some of which are very long; without scales. T.R. about 0·8.

Abdomen dark brown, well clothed with short hairs, without scales. Spermathecae two, moderately chitinised, oval, sub-equal, about 51μ by 43μ; the duct chitinised for only a very short distance, perhaps 2μ. The vulval orifice completely surrounded by a ring of chitin.

QUEENSTOWN, 11.ii.1922, 1♀ (L. Curtis); GOVERNOR'S BAY, 30.xi.1922 and 17.viii.1923, 2♀♀ (J. F. Tapley).

This species is allied to *F. australiensis* Kieff., which also has the wings unadorned and the legs banded, but the banding of the legs is less extensive, and there are a number of other differences; for example, the terminal segments of the antenna are longer, twice as long as the tenth segment, and the halteres are white.

Forcipomyia antipodum, Huds.

A dark brown species of moderate size, with unadorned wings, white halteres, dark or darkish brown legs the tibiae of which are armed with long lanceolate spines, and T.R. 1·7; without scales.

MALE AND FEMALE. Length of wing, 1·6 mm. to 1·9 mm.; greatest breadth of wing, 0·46 mm. to 0·6 mm.

Head very dark brown. Eyes bare, contiguous above, the facets separated by a narrow line. Palpi darkish brown; the third segment about as long as the fourth and fifth together, greatly inflated at the base, and furnished with a large sensory pit; the fourth and fifth segments cylindrical, fused together, but still showing

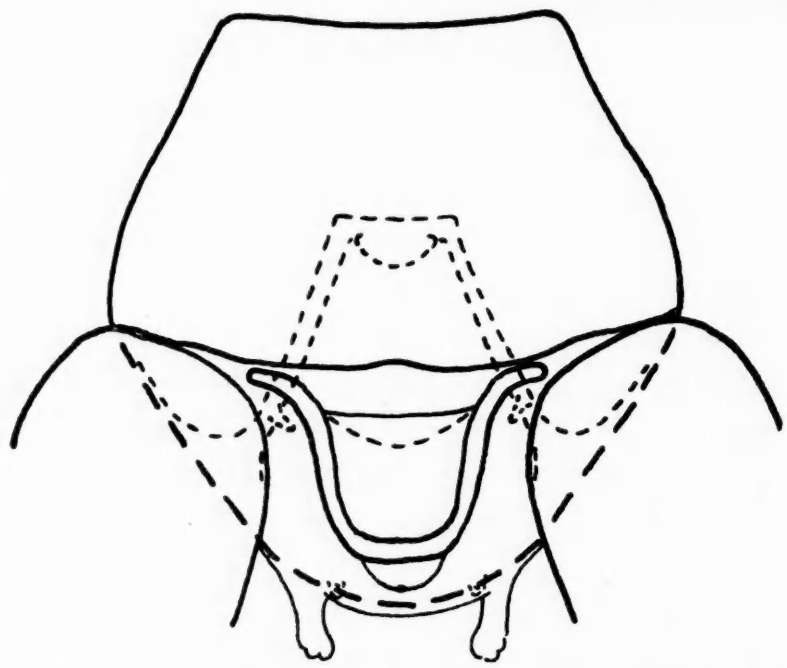

Fig. 1. *Forcipomyia antipodum*, Hudson: hypopygium of the male, ventral view.

a line of junction; the last three segments measuring approximately 28, 21, and 6 units respectively. Antennae brown in the male, dark brown in the female. In the male segments 4 to 11 measuring from 15 by 15 to 13 by 9 units; segments 12 to 15 elongated, 51, 39, 29, and 36 units respectively, the last segment terminating in a nipple-like process. In the female, segments 4 to 10 narrowed distally but not definitely flask-shaped, sub-equal, about 14 by 10 units, armed with long slender spines which are rather blunt; segments 11 to 14 sub-equal (20 to 22 units in length), about three times as long as broad; the last segment a little longer (29 units), terminating in a short process which is not constricted at its base. The combined lengths of segments 11 to 15, 4 to 10, and 3 to 10 approximately 118, 104, and 120 units respectively.

Thorax very dark brown; well clothed with hairs, those on the posterior part of the scutum being very long and overhanging

the scutellum. Scutellum very dark brown; bearing numerous bristles and hairs. Post-scutellum very dark brown, almost black.

Wings unadorned, without pale spots, but slightly infuscated; densely clothed with dark macrotrichia, but without scales. Venation as usual. Costa reaching to about the middle of the wing. First radial cell obliterated; second well formed, of the usual shape. Fork of Cu at about the level of the middle of the second radial cell in the female, at about the level of the end of the costa in the male. Halteres with white knobs.

Legs dark or darkish brown, with the knees paler brown than the rest as also are the extreme bases of the femora and the proximal tarsal segments. In the female the femora are almost uniformly dark brown, but in the male the distal portions are paler, especially on the four anterior legs. In the male the tibiae also are paler than in the female. Legs well clothed with hairs, but without scales. All the tibiae are armed with long, dark brown, lanceolate spines, which are more numerous in the female than in the male. T.R. about 1·7.

Abdomen very dark brown excepting the extreme tip and the cerci in the female, which are paler; without scales. Spermathecae two, highly chitinised, oval, sub-equal, about 90μ by 55μ; the duct chitinised for only a very short distance, about 3μ. Hypopygium of the male (fig. 1) very dark. The ninth sternite bearing numerous bristles (not shown in the figure), and with at most only a very small V-shaped excavation in the middle line posteriorly. Side-pieces and claspers more or less normal. Harpes similar to those of *F. ingrami*. Aedoeagus complex and rather variable in appearance according to the angle at which it is seen, in ventral view appearing as shown in the figure.

WHITE ROCK, 26.xi.1922, 1 ♂ (J. W. Campbell); OHAKUNE, v to vii.1923, 1 ♀ (T. R. Harris).

Thanks to the courtesy of Dr. F. W. Edwards, we have been able to compare these specimens with examples of *F. antipodum* presented to the British Museum (Natural History) by Dr. Hudson, and have no hesitation in identifying them as this species. In the original description of *F.* (*C.*) *antipodum* Hudson (1892) figured the adult insect, but gave no account of its morphology. We have therefore described the species so that it may in future be the more readily recognised.

Atrichopogon vestitipennis, Kieff.

A very dark brown, almost black species, with the scutellum not quite so dark as the scutum, the legs uniformly brown, the wings with numerous macrotrichia, and the halteres with whitish knobs.

MALE AND FEMALE. Length of wing, 2·0 mm. to 2·2 mm.; greatest breadth of wing, 0·55 mm. to 0·7 mm.

Head almost black. Eyes bare; contiguous above, the facets separated by a narrow line. Palpi very dark brown; the third segment about as long as the fourth and fifth together, inflated in

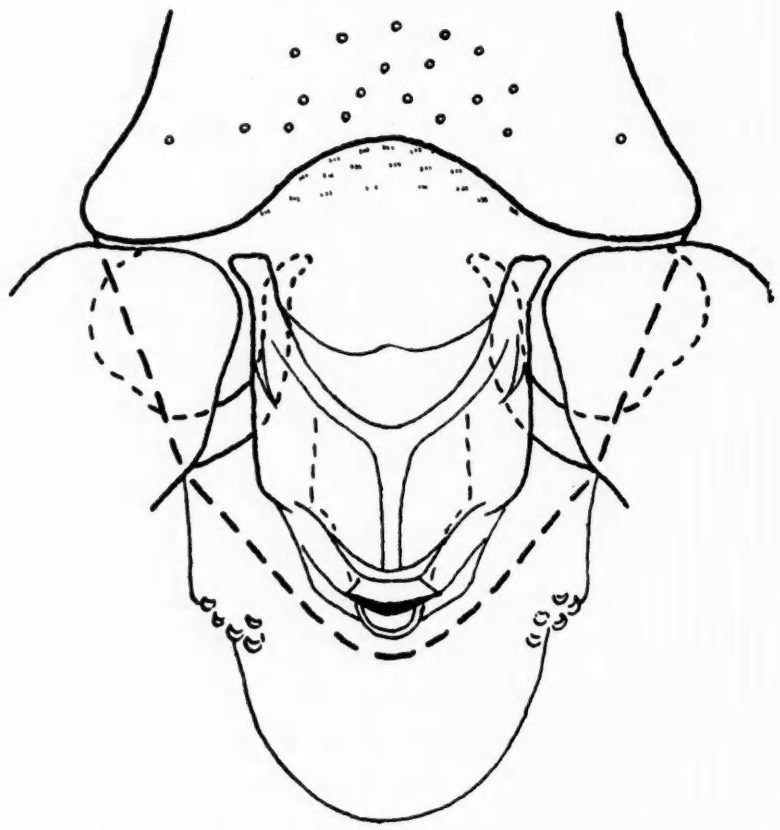

Fig. 2. *Atrichopogon vestitipennis*, Kieff: hypopygium of the male, ventral view.

the middle, with a large sensory pit. Antennae very dark brown. In the male, plume large and black; segments 4 to 11 as usual, measuring in one specimen from 16 by 15 to 14 by 11 units; segment 12 with a whorl of hairs, but drawn out distally; segments 13 to 15 elongated, about eight times as long as broad, the last segment ending in a rather long stylet. In the specimen measured the lengths of the last five segments were 14, 30, 53, 40, and 57 units respectively.

In the female, segments 4 to 10 sub-spherical, in one specimen measuring from 11 by 11 to 11 by 10 units; segments 11 to 15 longer, four or five times as long as broad, measuring in the same specimen 31, 30, 33, 35, and 43 units respectively, the last segment ending in a rather long stylet. The combined lengths of segments 11 to 15, 4 to 10, and 3 to 10 measured 174, 77, and 92 units respectively.

Thorax almost black. Scutellum not quite so dark as the scutum, and paler in the female than in the male; bearing the usual four bristles, and a number of small hairs. Post-scutellum almost black.

Wings of the usual form. Macrotrichia present in both sexes; in the female covering sparsely practically the whole wing surface excepting the radial areas and a line along each side of the veins; in the male limited to the tip, in cells R 5 and M 1. Costa extending about three-quarters of the length of the wing. First radial cell very narrow, slit-like; second larger, about three or four times as long as the first. Petiole of the median vein shorter than the cross-vein. Fork of Cu at about the level of the middle of the first radial cell. Halteres with whitish knobs and infuscated stems.

Legs uniformly brown, darker in the male than in the female. T.R. about 2·2. Forms of the segments, the claws, and the empodium normal.

Abdomen very dark brown, blackish. Spermathecae three, highly chitinised, oval, about 80μ by 60μ; the duct chitinised for some distance, about 30μ. Hypopygium (fig. 2) of the usual form. Ninth sternite bearing numerous bristles, broadly excavated in the middle line posteriorly. Claspers very dark, and rather thick. Aedoeagus in ventral view as shown in the figure.

OHAKUNE, xi.1922, 3 ♂♂, 6 ♀♀ (T. R. Harris); DUNEDIN, 1 ♂ (C. C. Fenwick).

This species is provisionally identified with *A. vestitipennis*, a New Guinea species described by Kieffer, which it clearly resembles closely. It should be noted, however, that Kieffer had only the female at his disposal, and that certain details, such as the form of the spermathecae and of the hypopygium, are missing from his description.

Dasyhelea, sp.

MALE. This insect, unfortunately, was so much damaged, almost the whole of the antennae and the abdomen being missing, that it cannot be adequately described. The characters which it was possible to make out show clearly that it belongs to a species hitherto undescribed from this region. The following points may be noted :—

Length of wing, 1·2 mm. ; greatest breadth of wing, 0·3 mm. Head almost black. Eyes densely hairy. Palpi very dark : the third segment not quite as long as the fourth and fifth together ; the fifth segment longer than the fourth. Antennae missing all but the four basal segments ; these were very dark brown. Thorax almost black. Scutellum almost black. Wings of the usual type. Costa reaching just beyond the middle of the wing. First radial cell obliterated ; second of the characteristic form, almost square. Fork of Cu at about the same level as the end of the costa or slightly distal to it. Halteres with very dark brown knobs, but containing a white substance which shows through at the tip of the knob. Legs uniformly very dark brown. Abdomen probably almost black, but only fragments of the two basal segments were left.

OHAKUNE, xi.1923, 1 ♂ (T. R. Harris).

Stilobezzia ohakunei, n.sp.

A yellowish-brown species, with a yellowish-brown scutellum bearing about four bristles, and rather pale brown legs.

FEMALE. Length of wing, 2·3 mm. ; greatest breadth of wing, 0·8 mm.

Head darkish brown. Eyes bare ; separated above by a wedge-shaped area. Mandibles armed with seven teeth. Palpi darkish brown, the segments sub-cylindrical, the lengths of the last three measuring in one specimen 22, 11, and 19 units respectively ; the third segment with a shallow, sub-apical depression bearing sensory hairs. Antennae dark brown, the segments sub-cylindrical ; segments 4 to 10 measuring in one specimen from 19 by 7 to 23 by 6 units, armed with long tapering spines ; segments 11 to 14 sub-equal, about six times as long as broad, their lengths in the same specimen 30, 30, 33, and 32 units ; the last segment a little longer,

38 units, without a stylet. The combined lengths of segments 11 to 15, 4 to 10, and 3 to 10 approximately 170, 140, and 165 units respectively.

Thorax yellowish-brown, the adornment as usual but indistinct, the shoulder areas, however, a little paler. Tuft above the root of the wing composed of about eight hairs. Scutellum yellowish-brown, bearing four bristles, and a few (4 to 6) small hairs. Post-scutellum darker brown.

Wings unadorned, of the usual form. Macrotrichia numerous at the tip, a fair number being present in cells R 5 and M 1 and 2, and a few between the rami of Cu and in the anal cell. Costa extending about four-fifths of the length of the wing. First radial cell rhomboidal, three or four times as long as broad ; second larger, three or four times as long as the first. Cross-vein and the distal part of R 1 not quite in line. Petiole of the median vein slightly longer than the cross-vein. Fork of Cu slightly proximal to the level of the cross-vein. Halteres somewhat infuscated, with brownish knobs.

Legs almost uniformly yellowish-brown. Segments normal, not swollen, unarmed. T.R. 2. First tarsal segment of the hind legs without a basal spine. Claws normal.

Abdomen dark brown. Spermathecae two (and a rudimentary third), highly chitinised, oval, but a little narrowed at the proximal end, sub-equal, about 90μ by 56μ ; the duct narrow, 3μ to 4μ, and chitinised for a short distance, about 8μ.

OHAKUNE, xii and i, 1922 to 1924, 3 ♀♀ (T. R. Harris).

Stilobezzia antipodalis, n.sp.

A very dark brown species, with a very dark brown scutellum bearing about nine bristles, and dark brown legs.

MALE. Length of wing, 1·8 mm. ; greatest breadth of wing, 0·46 mm. This unique specimen was rather badly damaged.

Head very dark brown. Eyes bare, widely separated above. Palpi darkish brown, the lengths of the last three segments 15, 10, and 15 units respectively, the third segment not inflated, with a shallow sub-apical depression bearing sensory hairs. Antennae dark brown, the plume large : segments 4 to 10 similar, oval,

measuring from 12 by 10 to 11 by 7 units ; segments 11 and 12 more drawn out distally, their lengths 12 and 16 units respectively ; segments 13 to 15 elongated, their lengths 38, 31, and 25 units respectively, and their breadths from 4 to 7 units, the last segment the broadest, not dilated at its base, without a stylet.

Thorax very dark brown. Scutellum very dark brown, bearing a transverse row of 9 bristles and about 8 small hairs. Post-scutellum very dark brown.

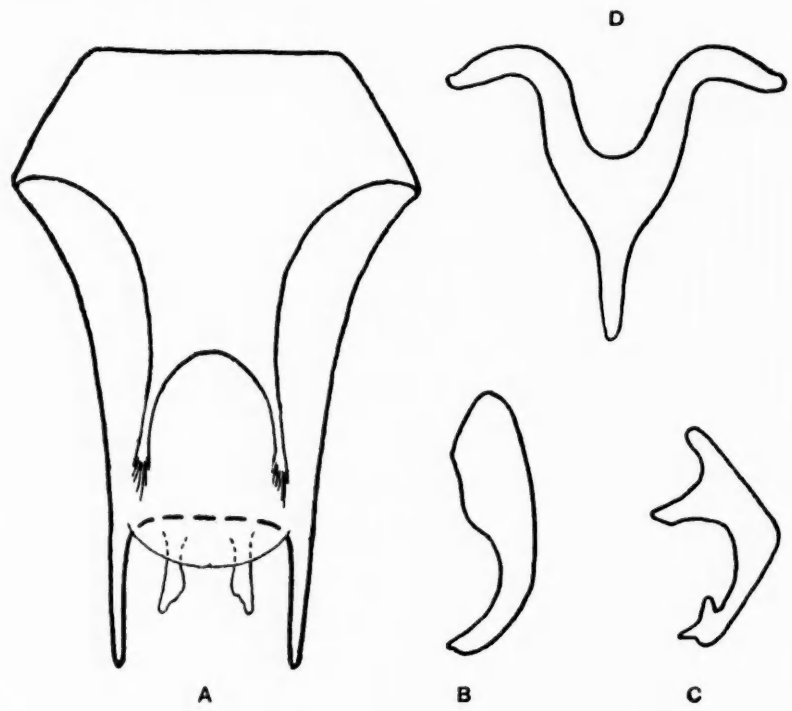

Fig. 3. *Stilobezzia antipodalis*, n.sp. : parts of the hypopygium of the male, ventral views. *A*.—ninth segment ; *B*.—clasper ; *C*.—harpe ; *D*.—aedoeagus.

Wings unadorned, of the usual form ; densely clothed with microtrichia which are minute, and bearing a very few macrotrichia at the tip, at the extreme periphery of cell R 5. Anal lobe poorly developed. Fringe short, of alternating long and short hairs. Alula with a fringe of hairs. Costa extending two-thirds of the length of the wing. First radial cell narrowed distally ; second larger, about once to once and a half the length of the first. Petiole of the median vein about as long as the cross-vein. Fork of Cu well distal to the level of the cross-vein. Intercalary fork not visible. Anal vein straight, but with a thickening about its middle. Halteres a little infuscated, with white knobs.

Legs almost uniformly very dark brown, but the tarsal segments

are a little paler than the femora and tibiae; well clothed with hairs and bristles, some of which are very long. Hind femora rather larger than the others. Femora and tibiae not armed with stout spines. Hind legs with the first tarsal segment armed with a basal and an apical spine, and bearing a longitudinal row of closely set spines running from base to apex. T.R. about 1·5. Fourth tarsal segment cordiform.

Abdomen very dark brown; hairs moderately long. Hypopygium (fig. 3) very dark. Ninth tergite bearing at its lateral angles posteriorly very long finger-like processes. Ninth sternite without bristles; prolonged posteriorly as two long slender processes with brush-like ends. Side-pieces short; claspers narrowing sharply about the middle. Harpes very highly chitinised, terminating distally in two prong-like processes. Aedoeagus Y-shaped in ventral view, the stem being somewhat gutter-like, and the arms widely arched laterally.

WHITE ROCK, 26.xi.1922, 1 ♂ (J. W. Campbell).

Monohelea antipodalis, n.sp.

A rather large, yellowish-brown species, with the scutellum yellow, the legs yellowish-brown with darker markings on the hind legs, white halteres, and a banded abdomen.

MALE. Length of wing, 2·5 mm.; greatest breadth of wing, 0·6 mm.

Head chestnut brown. Eyes bare, widely separated above. Palpi darkish brown, the segments cylindrical, the last three measuring in one specimen 27, 18, and 28 units in length respectively; the third segment not inflated, without a sensory pit, but bearing a few sensory hairs near the apex. Antennae darkish brown, the proximal segments a little paler, yellowish, at the base; segments 4 to 10 similar, oval, measuring in one specimen from 20 by 11 to 16 by 9 units; segments 11 and 12 more drawn out distally, their lengths in the same specimen 17 and 23 units respectively; segments 13 to 15 elongated, their lengths 60, 52, and 42 units respectively, and their breadths 7 or 8 units, the last segment not dilated at the base, without a stylet.

Thorax yellowish-brown, with the usual darker, chestnut-brown markings; without tubercle or spine. Scutal bristles rather long,

and dark ; there is a conspicuous tuft of about 17 just above the root of each wing. Scutellum pale, yellow ; bearing a transverse row of 12 dark bristles, and about 9 small hairs. Post-scutellum dark chestnut-brown.

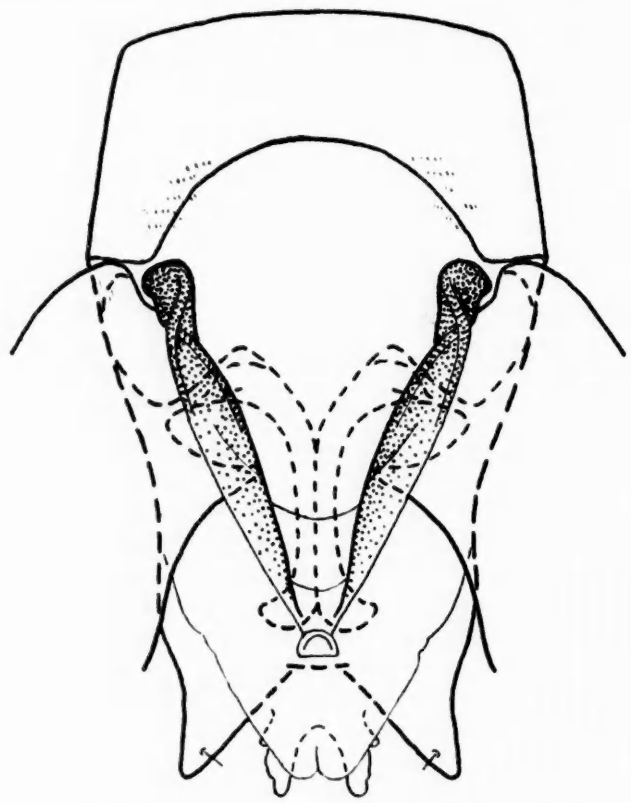

Fig. 4. *Monohelea antipodalis*, n.sp. : hypopygium of the male, ventral view.

Wings unadorned, the veins pale, yellowish ; densely clothed with microtrichia which are minute, and with a very few macrotrichia at the tip, at the extreme periphery of cell R 5. Anal lobe small, the angle very obtuse. Fringe composed of alternating long and short hairs. Alula with a fringe of hairs. Costa extending three-quarters of the length of the wing, not prolonged beyond its junction with R 5. First radial cell small, lenticular ; second large, rather over three times as long as the first. Petiole of the median vein about as long as the cross-vein. Fork of Cu slightly proximal to the level of the cross-vein. Intercalary fork not visible. Anal vein straight, but with a thickening about its middle. Halteres with white knobs.

Legs almost uniformly yellowish-brown, but the knees and the distal halves of the fifth tarsal segments are a little darker, as also are the hind legs which in addition are adorned with dark bands

just before the apices of the femora and at the apices of the tibiae. Legs densely clothed with dark hairs and long bristles some of which are strong and almost spine-like. Femora of the hind legs swollen ; the others normal. Femora not armed with spines of the sort found in *Palpomyia*. First tarsal segment on all the legs armed with spines not only at the base and apex, but also on the middle part of the shafts. Hind legs with the first tarsal segment curved and armed with three strong, black spines, one at the apex, one at the base, and one near the middle, and bearing a longitudinal row of closely set lanceolate spines extending from base to apex ; second tarsal segment armed with a single apical spine ; the other tarsal segments unarmed. T.R. about 2. Fourth tarsal segment of all the legs cylindrical, unarmed ; fifth long, curved, unarmed. Claws small, equal, about half the length of the last tarsal segment or smaller, with bifid ends. Empodium very small.

Abdomen with the proximal segments banded, dark and paler brown, the dark zones corresponding with the middle portions of the segments, and the distal segments almost entirely darkish brown ; well clothed with rather long hairs. Hypopygium (fig. 4) with the ninth segment and the aedoeagus yellowish-brown and the side-pieces dark brown. Ninth tergite bearing at its posterior margin triangular, chitinised processes. Ninth sternite bare, with a shallow excavation in the middle line posteriorly. Side-pieces normal ; claspers paler in the middle than at the ends. Harpes well chitinised rods which are contiguous at their inner sides, and have their ends slightly expanded and turned laterally. Aedoeagus V-shaped in ventral view ; the membrane joining it to the ninth sternite hardly at all spiculated.

OHAKUNE, xii and i, 1922 and 1923, 3 ♂♂ (T. R. Harris).

Palpomyia cantuaris, n.sp.

A large, very dark brown species with a small scutal tubercle, unadorned wings, dark brown halteres, the fore femora swollen and armed with about fifteen to twenty stout spines, and the fourth and fifth tarsal segments unarmed.

FEMALE. Length of wing, 3·3 mm. to 3·6 mm. ; greatest breadth of wing, about 1·0 mm. The species varies considerably both in size and colouring.

Head dark, reddish brown. Eyes bare, rather widely separated above. Mandibles armed with nine or ten long teeth. Palpi dark brown, the lengths of the last three segments in one specimen 26, 16, and 24 units respectively, the third segment sub-cylindrical, without a sensory pit, but bearing sensory hairs on its inner border. Antennae dark brown, the proximal segments paler at the base than at the apex ; segments 4 to 10 sub-cylindrical, sub-equal, measuring in one specimen from 18 by 9 to 18 by 7 units ; segments 11 to 15 elongated, slender, about 6 units broad, and their lengths in the same specimen 42, 43, 43, 44, and 51 units respectively (but in another 47, 46, 48, 51, and 45 units respectively), the last segment without a stylet. The combined lengths of segments 11 to 15, 4 to 10, and 3 to 10 measuring in one specimen 228, 127, and 158 units respectively.

Thorax in the dried insect dull brown, very dark excepting at the shoulders where it is a little paler. After treatment with carbolic, however, it is clear that the scutum is reddish-brown with very dark markings forming the usual pattern. There is a small scutal tubercle. Scutellum reddish-brown, about the same colour as the shoulders ; bearing six small bristles. Post-scutellum very dark.

Wings unadorned, but the anterior veins are often infuscated, especially in the neighbourhood of the cross vein ; without macrotrichia. Anal lobe well developed. Costa extending four-fifths of the length of the wing. First radial cell large, about five times as long as broad ; second about two and a half to three times as long as the first. Median vein with the distance from the fork to the cross-vein about equal to the length of the cross-vein. Fork of Cu at about the same level as the cross-vein, or slightly distal to it. Anal vein bent. Halteres with dark brown knobs.

Legs rather dark. Femora of the fore legs brown, with a dark brown band in the middle ; those of the four posterior legs sometimes entirely dark brown, but darkest at the apical third ; sometimes dark brown only at the apical half or one third. All the knee joints dark brown. Tibiae of all the legs with a dark band at the apex, usually somewhat infuscated at the base, and paler brown in the middle. Last two tarsal segments entirely dark brown ; third segment mostly dark brown ; and the first two pale

brown excepting at the joints. Femora of fore legs swollen and armed with numerous (about 15 to 20) stout spines; the other femora and all the tibiae slender and without such spines. T.R. about 2. Fourth tarsal segment cordiform, fifth not swollen; both unarmed. Claws normal, small, equal, barbed.

Abdomen very dark brown, especially posteriorly, the basal segments having a large paler brown area in the middle (but not reaching to the base) of the tergites. Spine-like supports for the eversible glands present on the tergites of the fourth or fifth to the seventh segments, and rudiments of them on the third and sometimes the fourth segments. Spermathecae two or three, highly chitinised, oval, in one specimen, sub-equal, about 100μ by 70μ; in another very unequal, from 60μ by 45μ to 110μ by 85μ; the commencement of the duct chitinised for a short distance, from about 5μ to 14μ.

SOUTH CANTERBURY, ii.1923, 3♀♀; OKAHUNE, xi to iii.1922 to 1924, 6♀♀ (T. R. Harris).

EXPERIMENTAL INFECTION OF ANTELOPES (DIK-DIK) WITH *TRYPANOSOMA RHODESIENSE*

BY

J. F. CORSON

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Bruce and his colleagues (1911) found that antelopes, experimentally infected with *Trypanosoma gambiense*, lived in captivity in apparently perfect health for at least four months. Duke (1913) showed that the blood of some of these antelopes was still able to infect laboratory animals from a year to twenty-two months later. The former authors mentioned the importance of the possible effect of infection on the health of antelopes in relation to their acting as a reservoir of the human trypanosome. As *T. rhodesiense* is usually so fatal to sheep and especially goats, quickly infecting the central nervous system, it seemed possible that it might also be virulent for antelopes. Some experiments were therefore made at Maswa, Tanganyika Territory, by direct inoculation of *T. rhodesiense* into small antelopes called Dik-dik, the only kind available at the time. These antelopes frequent the farms near houses and may easily be exposed to infection in some sleeping sickness districts. They were caught for me by natives in a fly-free area. Many died, chiefly adults, sometimes on the day of arrival and from no apparent cause. Others died from acute cellulitis arising from abrasions on the legs due usually to the string with which the natives tied their legs. They were kept in a roomy hut and fed readily on the green tops of sweet potatoes and the leaves of a leguminous bush, but this was probably not a sufficiently varied diet. The blood of about half of the animals showed the microfilariae of *Setaria hornbyi* or *boulengeri*, kindly identified by the Liverpool School of Tropical Medicine.

EXPERIMENTS

It was possible to carry out observations on four animals only as other infected animals died from various causes.

Dik-dik No. 1. Young adult female received on 26 November, 1930.

29.11.30. Blood, thick film, no trypanosomes and no microfilariae; inoculated with blood of Rat 119, strain 279 Wosuka.

Patient Wosuka — Case 279	Rat 84 inoc. 17.10.30	Rat 119 inoc. 23.11.30	Dik-dik 1 inoc. 29.11.30

10.12.30. Blood, no trypanosomes; a rat inoculated but did not become infected.

16.12.30. Blood, numerous polymorphic trypanosomes; inoculated into Rat 131 which showed polymorphic trypanosomes in its blood on 20.12.30, and posterior-nuclear forms in considerable numbers on 22.12.30.

20.12.30. The antelope was losing flesh rapidly and had sores on its legs but could walk about and fed well. Its blood showed no posterior-nuclear forms. Rat 132 was inoculated and showed trypanosomes in its blood on 24.12.30 and posterior-nuclear forms on 26.12.30. A thin blood film having been taken, the dik-dik was chloroformed to death, the suboccipital region dissected, cerebrospinal fluid withdrawn and examined with a Fuchs and Rosenthal counting slide. The fluid showed four living trypanosomes in a cubic millimetre but red blood corpuscles were present. The thin blood film showed five trypanosomes in 200 fields. It was therefore impossible to say that the cerebrospinal fluid was infected. Gland puncture just after death showed no trypanosomes.

Dik-dik No. 4. Young adult male received on 29 November, 1930; well-nourished and free from injuries.

29.11.30. Blood, thick film, no trypanosomes, microfilariae present.

10.12.30. In good health, fed well and had not lost flesh. Inoculated with blood of Rat 122, strain 279 Wosuka.

Patient Wosuka— Case 279	Rat 81 inoc. 17.10.30	Rat 122 inoc. 27.11.30	Dik-dik 4 inoc. 10.12.30

21.12.30. Considerable loss of flesh though active and feeding well. Blood, thick film, numerous polymorphic trypanosomes, about 10 per field (1/12 Obj.). Inoculated into Rat 133 which showed scanty trypanosomes in its blood on 24.12.30 and posterior-nuclear forms later. Dik-dik No. 28 was inoculated from this rat on 18.1.31.

29.12.30. Blood, thin film, trypanosomes about 1 in 3 fields; no posterior-nuclear forms. Prescapular glands enlarged.

3.1.31. Blood, thick film, trypanosomes about 5 per field; thin film, no posterior-nuclear forms.

8.1.31. Blood, thin film, trypanosomes 1 in 5 fields; posterior-nuclear forms present.

12.1.31. Blood, thin film, trypanosomes 1 in 3 fields; posterior-nuclear forms present.

13.1.31. Apparently well and active; prescapular glands smaller; chloroformed and the suboccipital region dissected and cerebrospinal fluid examined. It contained blood and showed trypanosomes and microfilariae. Lumbar puncture also failed to produce a specimen free from blood. Gland puncture showed no trypanosomes.

Dik-dik No. 11. Young male without horns received on 12 December, 1930; active and free from injuries.

13.12.30. Blood, thick film, no trypanosomes and no microfilariae. Inoculated with blood of Rat 124, strain 280 Maduru.

Patient Maduru— Case 280	Rat 124 inoc. 7.12.30	Dik-dik 11 inoc. 13.12.30

22.12.30. Thin but active and fed well. Blood, thick film, numerous polymorphic trypanosomes, about 40 per field. Inoculated into Rat 134 which first showed trypanosomes in scanty numbers on 31.12.30. Posterior-nuclear forms appeared on 8.1.31 and the rat died on 7.2.31 with numerous trypanosomes in its blood.

25.12.30. Blood, thick film, trypanosomes about 1 per field.

29.12.30. Blood, thick film, trypanosomes about 10 per field; thin film about 2 per field. Prescapular glands enlarged.

30.12.30. Blood, thick film, trypanosomes 50 per field; inoculated into Rat 138 which showed trypanosomes in its blood on 2.1.31; posterior-nuclear forms on 4.1.31 and died on 8.2.31.

3.1.31. Blood, thick film, trypanosomes 15 per field; thin film 3 per field; posterior-nuclear forms present; animal fairly well.

8.1.31. Blood, thin film, trypanosomes 4 in 200 fields.

12.1.31. Blood, thin film, trypanosomes 1 in 200 fields.

18.1.31. Blood, thin film, trypanosomes 1 in 5 fields.

27.1.31. Blood, thin film, trypanosomes 1 in 5 fields.

5.2.31. Blood, thin film, trypanosomes 1 in 200 fields. Glands smaller; animal in apparently good health but thin and with a dry coat.

20.2.31. Injected intramuscularly with 0·2 gramme of Bayer 205.

21.2.31. Blood, no trypanosomes; chloroformed and cerebrospinal fluid withdrawn by suboccipital puncture and examined. It contained red blood corpuscles; no trypanosomes were seen.

Dik-dik No. 28. Very young male without horns received on 28.12.30; well-nourished and active; slight sores on the legs.

28.12.30. Blood, thick film, no trypanosomes, numerous microfilariae.

18.1.31. In good health; inoculated from Rat 133, infected from Dik-dik No. 4.

27.1.31. Blood, thin film, polymorphic trypanosomes present, about 2 per field.

5.2.31. Blood, thin film, trypanosomes 1 or 2 per field; no posterior-nuclear forms; animal in good health; leg sores healed.

19.2.31. Blood, thin film, trypanosomes 4 in 200 fields. Prescapular glands enlarged; animal in apparently good health but with dry coat.

20.2.31. Injected intramuscularly with 0·15 gramme of Bayer 205.

21.2.31. Blood, trypanosomes present.

22.2.31. Blood, thick film, no trypanosomes. Chloroformed and cerebrospinal fluid withdrawn by suboccipital puncture. The fluid contained no cells and no trypanosomes.

SUMMARY AND COMMENTS

Four antelopes, dik-diks, were inoculated with *T. rhodesiense* and proved to be readily susceptible. Trypanosomes were numerous in the blood and posterior-nuclear forms were present. The lymphatic glands became enlarged but gland puncture showed no trypanosomes. No evidence was obtained of infection of the central nervous system. The animals appeared to suffer little in general health during a period of about one month. In sheep and goats severe wasting is soon evident and numerous leucocytes and trypanosomes can be found in the cerebrospinal fluid, often within a month from the date of inoculation. It does not appear that even selected resistant sheep may be used in experiments to represent antelopes as regards the question of a reservoir as the objection that sheep are not antelopes is unanswerable. These experiments were made mainly to see if the central nervous system became infected ; circumstances unfortunately curtailed the period of observation. No attempt was made to observe closely the behaviour of the trypanosomes in the antelopes.

Observations over a longer period and under better health conditions than were possible at Maswa and on a larger number of animals are needed. Transmission by tsetse flies is of course required to get a closer resemblance to what may occur in Nature.

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CONTRIBUTION TO THE STUDY OF THE PATHOLOGY AND MORBID HISTOLOGY OF HUMAN AND BOVINE ONCHOCERCIASIS

BY

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INTRODUCTION AND HISTORICAL REVIEW

The morbid histology of human and bovine onchocerciasis has been studied by many workers, but on reviewing the literature a student of the subject fails to find any constant picture of the microscopic manifestations produced in these diseases. The descriptions given by various pathologists do not supplement each other to form a definite pathological entity. This is believed to be due to the fact that the histological picture produced as a result of the tissue reaction against this helminthic invasion exhibits more than one phase or stage, so that the lack of uniformity of the histological pictures given might be due to the fact that the different

authors described the one or more stages which they happened to find in their material. Even those workers who have described many pathological manifestations have not attempted to explain them or to link them up to form a complete picture. This difficulty arose, the writer believes, from the lack of attempts to explain the genesis of the pathological manifestations produced, especially the formation of tumours.

The writer proposes first to give a short description of the pathological findings of some of the workers, next to describe his own findings in the material studied and lastly to discuss the whole subject.

Labadie-Lagrave and Deguy (1899) in discussing the genesis of the tumours of *Onchocerca volvulus*, stated that they examined a case of a French soldier in which the immature female worm was lying free in a dilated inflamed lymphatic vessel surrounded by a fibrinous exudate containing numerous leucocytes and phagocytes.

Prout (1901) examined a tumour from a frontier policeman at Sierra Leone which was about one inch in diameter by three-quarters of an inch in depth. On making an incision, a greenish semi-purulent looking fluid about the consistency of cream escaped out of the cyst. This, on microscopical examination, was found to contain numerous filarial embryos. The capsule of the cyst consisted of dense fibrous tissue, lined internally by a layer of soft caseous looking material which could be easily scraped off. This was composed of granular material, flat nucleated epithelial cells and contained free embryos. The interior of the cyst was filled with the adult worms, lying in loops, and twisted up in the most confusing fashion, entering the cyst wall, running along shallow tunnels and re-entering the cyst. Owing to this arrangement and the softness and friableness of the worms, it was a matter of the greatest difficulty to dissect out the worms without breaking them. He was, however, able to isolate from this tumour, one complete unbroken male and the head and tail and intermediate fragments of a female.

Brumpt (1904) agreed with Labadie-Lagrave and Deguy that an inflammation occurs in the lymphatic vessel in which the worm lies and further stated that the resulting exudate surrounding the worm is later organized into fibrous tissue. In older tumours a lympathic vessel could no longer be recognised as a point of origin

of the tumour formation. He also commented that the tumours always occur in situations rich in lymphatics.

Fülleborn (1908) stated that he could neither prove nor disprove the above-mentioned genesis of tumour formation of Labadie-Lagrave, Deguy, and Brumpt. In describing the pathology of the tumours, he noted that a section of the tumour showed a dense fibrous outer layer and in the interior a slimy pus-like greenish and sometimes reddish mass. This mass lay in a fibrous network in which sections of the worm were seen. Other sections of the worm were found in the fibrous tissue of the outer layer. The filariae in young tumours lay in a mass without structure, appearing scaly in sections and containing numerous leucocytes. This mass was gradually organized from the periphery inwards, into a firm fibrous tissue containing capillaries and thus enclosed the worms in canals. In older tumours the main mass consisted of a fibrous tissue very rich in cells. In apparently still older ones, the tissue around the worms was partly liquefied, so that a formative and a destructive process appeared to balance each other. Thus in the old as well as in the young tumours one saw parts in which the worm sections were lying in amorphous masses rich in leucocytes, if the contents had not fallen out in sectioning. These masses in the old tumours contained numerous larvae, but none were found in the younger tumours. Parsons (1908) stated that he met with five cases in Northern Nigeria exhibiting tumours of *Onchocerca volvulus* varying in size from a split pea to a hen's egg. These tumours were freely movable over the sub-adjacent tissues, but were more or less firmly attached to the overlying skin. In older tumours there might be very slight mobility and the tumours might even be adherent to the underlying bone. The tumours were very chronic in character and suppuration was not met with. As a rule the excised growth was found to consist of two or three tumours of varying sizes, all closely invested with a strong dense fibrous capsule. On section, the peculiar testicular feeling on palpation was explained. An opaque grumous fluid exuded from the tumour which was seen to be composed almost entirely of an inextricable network of worms. These were closely interwoven in the cortex of the tumour, while usually there was a central cavity which was bridged in every direction by the more or less disengaged portions of the male and

female worms. The worms lay embedded in the fibrous stroma which was probably formed as a result of reaction against the presence of the worms, and the fact that the adult worm had a rough and transversely corded cuticle, made it easy to account for the irritation that is set up, or it might be that the fibrosis was the result of a secretion or excretion on the part of the worm. The fluid found in these tumours seemed to vary according to the age of the tumour. In young tumours the fluid was scanty and of semi-opaque, milky appearance. In older and larger tumours it became thicker and more opaque and took on a dirty yellowish tinge. It might even assume a reddish colour. Microscopical examination revealed that the fluid of these tumours contained ova and embryos in large numbers together with fatty particles and cellular debris.

Ouzilleau (1913) stated that in the region of Mbobu one of the two head waters of Ubangi, the proportion of elephantiasis in different districts varied from 0·3 to 3 per cent. The different forms comprised elephantiasis of the scrotum and penis, labia majora and minora, the inferior extremities, lymph scrotum and adenolymphocele. The human filariae found, according to the author, were of three species, viz., *Filaria perstans*, *Filaria loa* and *Filaria volvulus*. *Wuchereria bancrofti* was never seen by the author in 1,500 day blood examinations nor in 400 night ones. The endemic index of *F. perstans* was 64 per cent. in natives above the age of 10 years. The index of *F. loa* was much less, 16 per cent. These two parasites were frequently found in the same blood and the patients might suffer at the same time from cysts of *F. volvulus*, though it would appear that an infection by the latter exercised an unfavourable influence on the two former. *Filaria volvulus* seemed to be very common. The cysts in which the parasite lives, were generally superficial and situated under the skin, which was movable over them. Adhesions, however, often bound them to the surrounding tissues or to the muscles and often even to the bone. They were generally very apparent and were almost always situated on the lateral aspects of the thorax. Sometimes they were very large, about the size of an apple, and then they were apparent at a glance in the semi-naked natives. They varied considerably, however, in volume, some being the size of a pigeon's egg or much smaller. Less frequently he found cysts on the osseous surfaces or over the joints in contact with the

skin. In addition he found them on the inner parts of the inguinal folds, over the symphesis pubis and along the cord, that is, in areas where they would be easily confused with lymphatic glands. He rarely found embryos in the blood.

He stated that the tumours which occur around the joints, could not at first sight be distinguished from those tumours which are known as juxta articular nodules, but every time he found these nodules he punctured them, and each time found *Microfilariae volvulus* and often fragments of adults. He also found the embryos in a large percentage of his cases in the enlarged gland masses of the crural region and in the lymphatic network of the inguinal crural area. He found that all cases of elephantiasis or bearers of similar tumours were infected with *Filaria volvulus*. This infection was diagnosed by the simultaneous presence of adult volvulus in the cysts and of embryos which were usually found in the concomitant inguinal adenitis or else by one of those alone. Thus in seventy-nine cases of elephantiasis, adenolymphocele and lymph-scrotum, thirty-five bore definite volvulus tumours and inguinal adenitis with microfilariae in the glands, and forty-four had no apparent tumours but had inguinal adenitis with embryos in the glands. He did not find a single case with cysts only.

He thus concluded that every case of elephantiasis or similar tumour was infected with *Filariae volvulus*. He questioned whether inversely every case of volvulus was a bearer of elephantiasis and to answer this question, he stated that :—

(1) Not every case of volvulus was necessarily a case of elephantiasis and some showed no tendency to develop elephantiasis.

(2) The volvulus patients always had inguinocrural adenopathy with microfilariae, this being always simple adenitis which sometimes became adenolymphocele.

(3) This adenopathy could exist and persist a long time without interfering with the health of the patient, but often complicated by general and local troubles of importance.

He lastly suggested, because of the similar geographical distribution of *F. volvulus* and elephantiasis in this region of Africa and because of the constant presence of volvulus in cases of elephantiasis in the same region, and because of the preference of the embryos for the inguinal and crural lymph glands, that *Filaria volvulus* was

to be incriminated as the pathogenic cause for local elephantiasis, equally with *F. bancrofti* for elephantiasis of the Arabs. The mode of action, he said, did not seem to be identical with that suspected by Manson for *W. bancrofti*, for the embryos are always present in the lymph glands of cases of volvulus elephantiasis and are always found there alive without the egg membrane (while the embryos of *F. bancrofti* disappear from the blood in elephantiasis according to Manson). The lymphatic stage here according to Ouzilleau is thus due to living embryos (while in bancrofti filariasis it is due to the eggs), and it would be the result of inflammation of the lymphatic glands and of the sclerous transformation through which they pass in the presence of the embryos owing to mechanical, irritative and toxic properties. He then commented that if in the secondary phenomena of inflammation or lymphangitis microbic infection with streptococci, staphylococci or gonococci, could have an influence in certain attacks of elephantoid fever, there was no need to attribute that to them always, the filaria infection being quite sufficient to cause the local inflammation and the general febrile condition.

Regarding the distribution of the embryos, he stated that the serum of the cysts was the most common place to find them and next to this was the lymphatic glands of the groin. Here the glands sometimes enlarged to the size of a hen's egg. He found them in other glands but this was rather exceptional. He also found them in adenolymphoceles.

Dubois (1916) during a journey through the Welle district was able to confirm many of the observations on *Onchocerca volvulus* made by Ouzilleau. He stated that the parasite was extremely common in that district especially in the vicinity of the important waterways. He noted also that *Wuchereria bancrofti* was unknown and that chylous hydrocele and haematochylurea were also unknown, He examined a series of 1,449 infected cases to determine the relative frequency with which the volvulus tumours occupied the various locations cited below and his results were :—around the trochanters, 30 per cent. ; iliac crest, 29 per cent. ; sides of thorax, 21 per cent. and mixed (i.e., situated in two or three of the preceding sites or elsewhere), 19 per cent. He considered that in order to determine the endemic index of a locality, examination of the three sites given would yield a sufficiently accurate result for practical

purposes. In the cases in which the tumours could not be found, the infection might be determined by the presence of the embryos in the fluid obtained by puncture from the more or less engorged inguinocrural glands. He stated that the embryos were present regularly in the lymph of these glands, but less frequently established in the cervical glands. He did not find them, however, in the blood.

In the case of fifty-three patients suffering from elephantiasis he found thirty-eight carriers of *Onchocerca volvulus* tumours; eleven others were determined to be infected by puncture of the groin glands, and in four only was the presence of *O. volvulus* not established. He thus considered that all cases of elephantiasis in the Welle district were infected with *O. volvulus*. Keratodermia was especially frequent amongst carriers of volvulus tumours in heavily infected districts. It sometimes co-existed with elephantiasis, and he saw typical cases of it in children. The incidence of volvulus tumours was very variable in different localities, but the heaviest infection was found on the Welle where from 50 to 60 per cent. of the adult population, and from 15 to 20 per cent. of the children were carriers of volvulus tumours. Cases of elephantiasis and keratodermia abounded here also.

In 1917 Dubois again stated that in his last paper he reported on the distribution of *O. volvulus* in Bas-Ouellé and this time he had visited the Haut-Ouellé and the information collected confirmed generally the results of Ouzilleau. He met with forty-eight cases of genital elephantiasis, forty-three of which had volvulus tumours, and four were positive by puncture of the inguinal glands. Only one case was negative for both. He saw elephantiasis of the foot in four other cases which also exhibited tumours. Keratodermia was commonly seen in the infected zones but juxta articular nodules were very rare. He thus concluded that among the riverine population of the Ouellé-Bomou basin a form of elephantiasis is associated with *F. volvulus* infections and is not to be confused with that resulting from *W. bancrofti*.

Calderón (1917) reported a paper read by Robles before the Società Juventud Medica in March, 1917, in which he stated that the latter had come across patients in Guatemala who exhibited tumours on the head which were associated with eye symptoms and an erysipelas-like condition of the face. The symptoms were

different in the acute and chronic stages of the disease. In the acute stage the whole face was swollen, the skin red, tense and shining, and the temperature rising to 104° F. In children the conjunctivae were inflamed, the ears, eyelids and lips were swollen. The patients complained of sharp pain all over the affected skin and of subjective sensations as if insects were crawling over the face. The cornea presented a condition of keratitis punctata, and iritis was a further complication in some cases.

In the chronic stage, the cheeks exhibited a hard oedema, the skin was shining with a livid greenish colouration, the ears were doubled in size with furrowed and scaly integument. If the limbs were affected there was a uniform hard oedema resembling that of elephantiasis of the Arabs. The cystic tumours associated with the disease contained a filaria identified as *Filaria volvulus*. On complete removal of the cysts with their fibrous prolongations, the symptoms cleared up quickly.

Calderón (1920) stated that the American Onchocerca in Guatemala predominated at heights between 1,800 and 3,600 feet above sea level where, on some estates, over 70 per cent. of the inhabitants were infected. The tumours varied in number from one to nineteen, and were almost always found on the head, almost invariably above the aponeurosis. Their situation indicated in his opinion, that the infection was transmitted by some nocturnal biting insect. The condition set up was of an erysipelas-like nature though suppuration did not occur. As a rule the swellings were painless and erysipelas only arose in tumours containing both males and females, and he believed that the acute stage was due to the discharge of embryos into the circulation, though he was unable to verify this. At the onset there was a rise of temperature but no enlargement of the lymphatic glands. The features were swollen and discoloured and the eyes closed partly from swelling and partly from photophobia. Within seven to ten days after excision of the incriminated tumour all the symptoms cleared up. Some cases were chronic from the start. The skin became gradually thickened with the formation of rugae and of a pallid greenish tinge with oedema. Exacerbation of these symptoms might occur at intervals of one to two months. Eye symptoms commonly associated were punctate keratitis, corneal leucoma, acute or chronic iritis, pigmentation of the sclerotics and impairment of vision. These symptoms were said to be due to a

toxin liberated by the parasites. There was a marked eosinophilia of 50 per cent. or over.

Montpellier and Lacroix (1920) found among native troops from Africa a number showing a skin infection or itch occurring especially on the buttocks, sides and lumbar region. With this was frequently associated inguinal, and also crural swellings and eosinophilia varying from 5 to 40 per cent. Systematic examination resulted in finding the constant presence of microfilariae in the dermal layer of the skin independent of the vascular network. These were considered to be the embryos of *F. volvulus* or a nearly allied species. The affection was regarded as identical with craw-craw as described by O'Neil (1875).

Further investigations showed the occurrence in some patients of fibrous cysts, one of which upon dissection yielded a male and part of a female, *O. volvulus*. Filarial itch was thus considered to be a dermal manifestation of onchocerciasis.

These authors with Degouillon (1920) upheld their above statement regarding filarial itch or craw-craw. So far as their researches had gone craw-craw or filarial itch corresponded in its geographical distribution with *O. volvulus*. Except in rare instances all the patients under observation showed fibrous tumours of onchocerca, and further no single carrier of these tumours was without the eruption characteristic of filarial itch.

Brumpt (1920) stated that the whole collection of biological facts made him doubtful of the rôle of *O. volvulus* in the causation of filarial itch.

Rodhain (1920) in speaking of human onchocerciasis in the Belgian portion of the Congo basin stated that the tumours occurred very generally among the dwellers along the main river and its principal tributaries. He found tumours which were filled with pus mixed with blood. Suppuration was usually limited to the interior of the tumours, the latter in some instances being partly destroyed with more or less complete liberation of the parasites. He came across a child bearing a cyst on the occiput. There occurred in addition some slight swelling of the face, puffiness of the features and dryness of the hair which entirely disappeared when the cyst was removed. This seemed to show that the cyst was the cause of the skin trouble of the face and head.

The majority of the tumours did not adhere to the skin, in others

there was undoubted adhesion. In only one instance did the author see a possible invasion of a lympathic gland.

Montpellier and Lacroix (1921) stated that the study of 150 cases of filarial itch in Colonial troops showed that the disease took the following course. First an intense itching of the affected area with no visible macroscopic or microscopic lesions. A section of the skin taken at this time showed an invasion of microfilariae while none were present a few days previously. The patients scratched violently, and in some cases showed urticaria or transient itching papules. In a few days the itching subsided, the skin became normal and sections of it showed no microfilariae. A few weeks or months later another attack occurred, and successive attacks produced finally permanent skin lesions. This stage was termed ' la période d'état ' ; there were diffuse lesions of tender, rough, thickened skin, and in places a dry papular eruption scattered over with papulo-vesicular pustules. Diagnosis at this stage was easy and skin sections almost always showed larvae, generally in large numbers, but they were not found in the head of the papules, hence superficial scarifications might not show any. This was attributed to their living outside the vessel in the surrounding tissue. In the early stages diagnosis was not easy, but sections of the skin of patients having an onchocerca tumour should be carefully examined during the itching stage.

The absence of larvae in onchocerciasis was rare and was attributed to the presence of a female worm only. The varying degree of the affection was typical of prurigo and was due to individual variations of susceptibility of the skin. The apparent absence of periodicity might be increased by the presence of several adults which would necessarily produce an intermittent discharge of embryos. At any given moment a patient having an adult *O. volvulus* might present no symptoms of filarial itch but it was quite the exception in the opinion of the authors, for this condition to be permanent.

Montpellier, Degouillon and Lacroix (1921) examined 800 Senegalese native troops ; 156 of these were carriers of mother tumours of *Onchocerca volvulus* or attacked by filarial itch. 150 of the 156 were of the latter class, and only six of the 156 though carrying mother tumours had no skin eruption. The inference was

therefore drawn that the presence of Onchocerca determines the eruption.

Montpellier, Lacroix and Boutin (1921) examined systematically the blood of natives infected with *O. volvulus*, some were examined whilst suffering from an eruption caused by the parasites and others during an intervening period. They did not find embryos in the blood films taken from the patients. They found that the percentage of eosinophiles varied from 0 to 70, and stated that the maximum eosinophilia seemed to occur at each discharge of microfilariae 'into the blood,' i.e., at the beginning of each skin eruption and the minimum to correspond with each period of microbic infection due to scratching, i.e., towards the end of each eruption.

Ouzilleau, Laigret and Lefrou (1921) described a region along the right bank of the Congo where *O.volvulus* infection was very common. Some patients exhibited cutaneous lesions in addition to cysts and one had keratitis. The cutaneous lesions were elephantiasis pseudo-icthyosis, ichthyosis simplex and lichenization. In describing the histo-pathological lesions they stated that there was an infiltration of connective tissue and mononuclears in the dermis and microfilariae were found in it. In the places where pigment was lacking a dense connective tissue was found which contained neither vessels, glands nor larvae. In the epidermis general hyperkeratitis was noticed often with infiltration of the stratum germinosum and stratum filamentosum with leucocytes. In the lichenozed zones, enlarged dome-like papillae were found. In the regions of the prurigo similar lesions were found, as described above, with blisters in addition which completely destroyed the epidermis. In the leucodermic areas there was complete absence of pigment and the horny layers were much thinner than usual. In the cysts microfilariae were found only rarely, in the elephantiasis lesions, parakeratitis existed, papillary vessels were very dilated, microfilariae swarmed in the dermis and spread into the hypodermis. Sebaceous glands were very rare and sudoriparous glands were entirely absent. From the above data they drew the following conclusions :—(1) That subjects infected with *O. volvulus* always had microfilariae in the dermis. (2) In their opinion craw-craw was not connected with onchocercal disease. (3) They stated tentatively that the cutaneous lesions were not caused through pruritus, but that the microfilariae created

inflammatory reactions in the skin which manifested themselves by pseudo-ichthyosis, elephantiasis of the genitalia and other parts, by achromy and cutaneous atrophy.

Ouzilleau (1923) found on examination of a large number of the natives in the Congo that some 40 per cent. were harbouring *O. volvulus*. In Upper Oubangui all cases of elephantiasis observed (79 in number and 3 per cent. of the whole) were similarly parasitised.

Microphotographs of sections of elephantoid skin were reproduced and showed the microfilariae of *O. volvulus* in the papillary and subpapillary layers.

Other conditions ascribed to oncherciasis were a cutaneous pseudo-ichthyosis and lichenisation, possibly craw-craw, some forms of keratitis and various arthropathies and nutritional disturbances.

Castellani (1925), in speaking about *O. caecutiens*, said that the tumours in a well-marked case were numerous, each the size of a pea to a large nut. They were seen on the scalp, though less frequently they might be found also on the body, especially on the sides of the chest close to the ribs. The nodules were deeply embedded in the subcutaneous tissues and the skin over them was often movable. He is inclined to believe that erysipelas of the coast and the eye troubles that are frequently associated with the presence of tumours are due to the presence of the latter. He is also inclined to believe that the American and African volvular diseases are not identical. He reproduced a microphotograph of a tumour section which showed a fibro-cellular tissue with microfilariae lying free in it.

Sharp (1926) stated that at Kaduna in Nigeria onchocercal tumours were frequent. In 90 per cent. of infected cases the tumours were found on the chest below the axilla or immediately above the anterior superior spine of the ilium on either side. Of the remainder most occurred in relation to the joints simulating juxta articular nodules. No tumours were found on the head. The tumours frequently had a long history of many years and were not met with in children. Europeans were not infected.

As a result of skin examination for *O. volvulus* embryos on one hundred prisoners he found 55 per cent. harbouring larvae in the skin and of these only 30 per cent. exhibited tumours. Of the infected patients some had hydrocele, others showed marked

thickening of the skin, others exhibited every variety of craw-craw and lichenification, while in the majority the skin was clean, smooth and healthy in appearance.

The embryos were seen in the skin in the various layers of the cutis vera up to the apices of the papillae and in the great majority of cases they caused no visible pathological changes, but under certain conditions they seemed to produce definite inflammatory phenomena. In such cases there was an inflammatory change around the capillaries, the walls of which were thickened, first by swelling of the endothelial cells and secondly by a cellular infiltration of lymphocytes around the capillaries. He explained this inflammatory change around the vessels by the fact that in one patient in whom it was found, microfilariae were found in the blood and this filariaemia produced a pseudo-septicaemic condition and ultimately the inflammatory changes.

Blacklock (1926) found *Onchocerca volvulus* infection very common in the Konno district of the Protectorate of Sierra Leone. He found larvae of this parasite in the skin of 45 per cent. of persons examined systematically. About the larvae in the skin and nodules of the patients he found that only 40 per cent. of those who had larvae in the skin had nodules also, while of those who showed no larvae in the skin 16 per cent. only had nodules, and so the examination of the skin gave a higher percentage of positive results. He also found statistically that neither in the case of the eyes, skin or glands did the patients show pathological effects solely and definitely due to *Onchocerca volvulus* infection. He also examined systematically the skin of a number of patients at different regions and found that the waist region was the one in which the larvae were most commonly met with.

The nodules varied from smooth soft, just palpable nodules to a large irregularly nodular hard mass of the size of a walnut. He recorded cases where larvae were found in the skin while the nodules which were present gave a negative result on puncture. Other cases showed the larvae in the skin and nodules but no cases were met with in which larvae were present in the nodules and absent in the skin. He commented that if the region had not been one in which *O. volvulus* was present then several of the cases would have been classed as juxta articular nodules. He quoted two cases,

FIG. 1. Camera lucida drawing of a section in the *O. volvulus* tumour, showing the worm above surrounded by a tunnel (part of it is seen below), which shows early proliferation of endothelioid cells and some giant cells and polymorphs. × 275.

D. Dagnall, del.

FIG. 2. Shows the *O. volvulus* worm to the left, and part of the tunnel wall to the right, which is made up wholly of endothelioid and some giant cells, with a moderate number of eosinophiles. × 275.

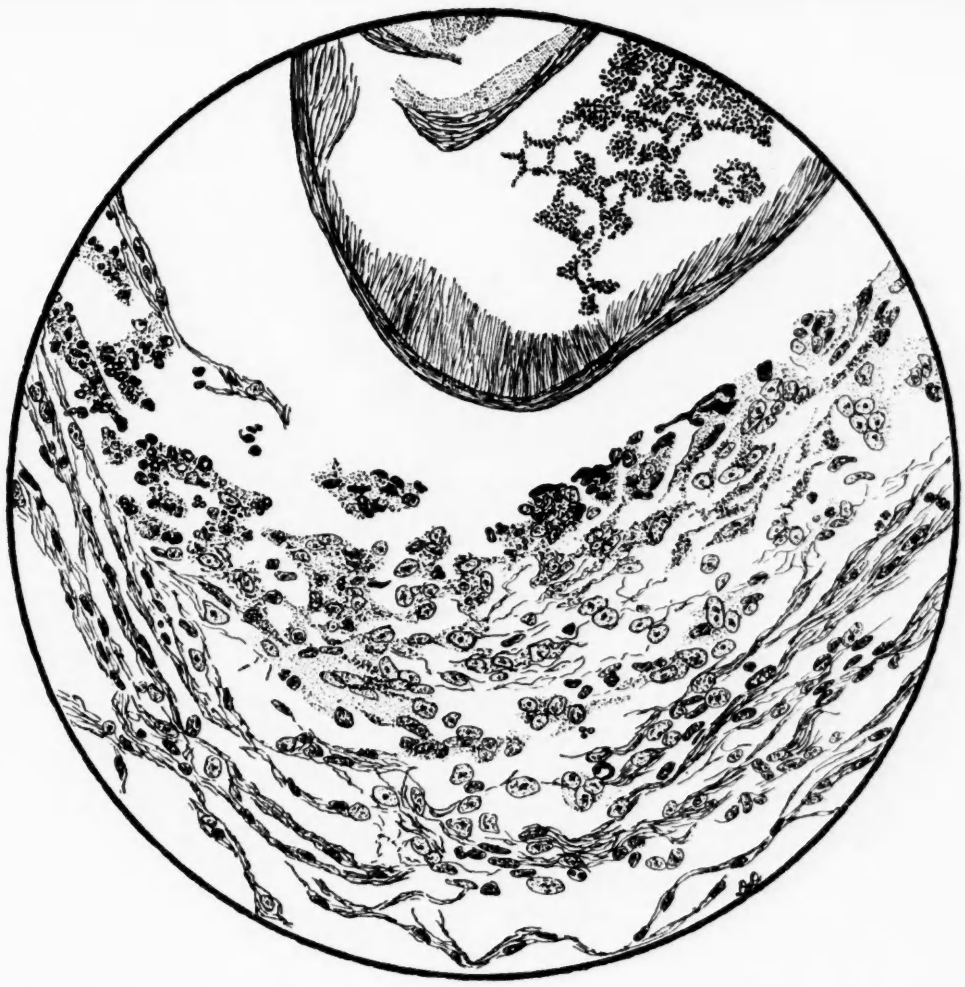


Fig. 3. Shows the worm above, surrounded by a fibro-cellular tunnel wall below, which is made up of cellular elements next to the worm and the process of fibrosis is advancing from the periphery inwards. × 275.

D. Dagnall, del.

Fig. 4. Shows the tunnel wall to the left made up of adult fibrous tissue bundles with fibroblasts in between. × 275.

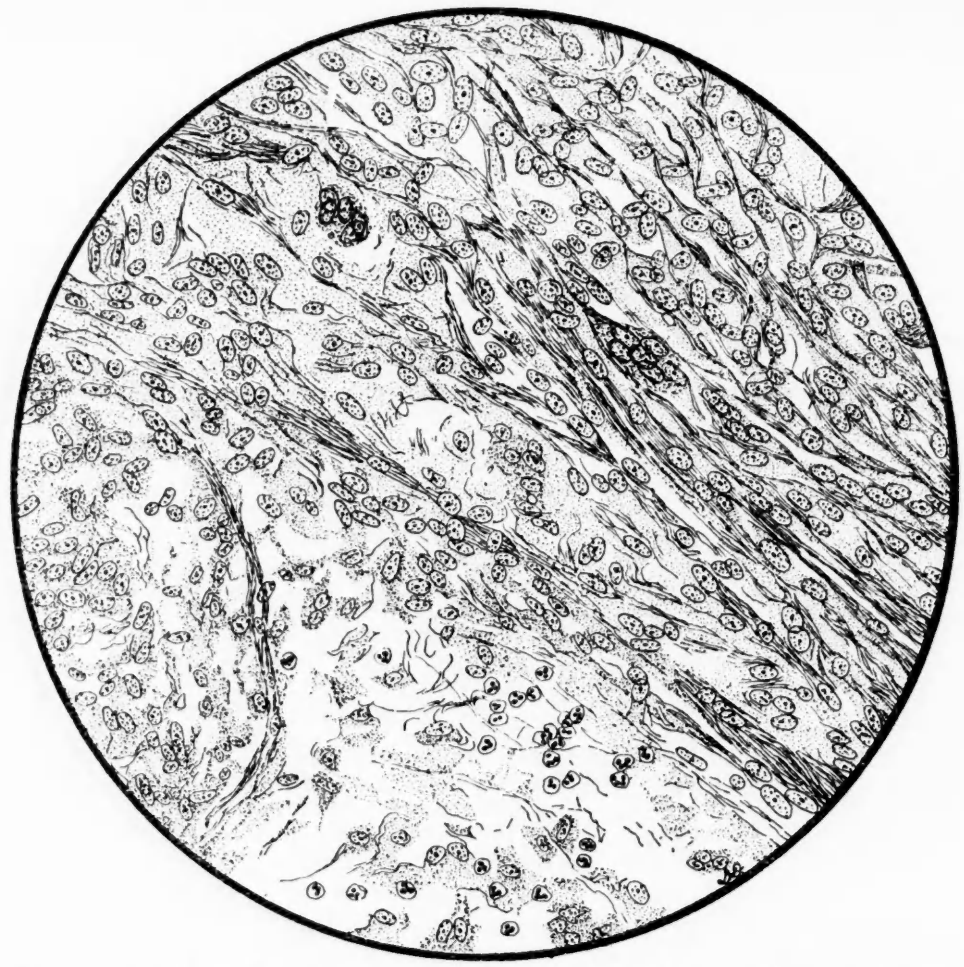
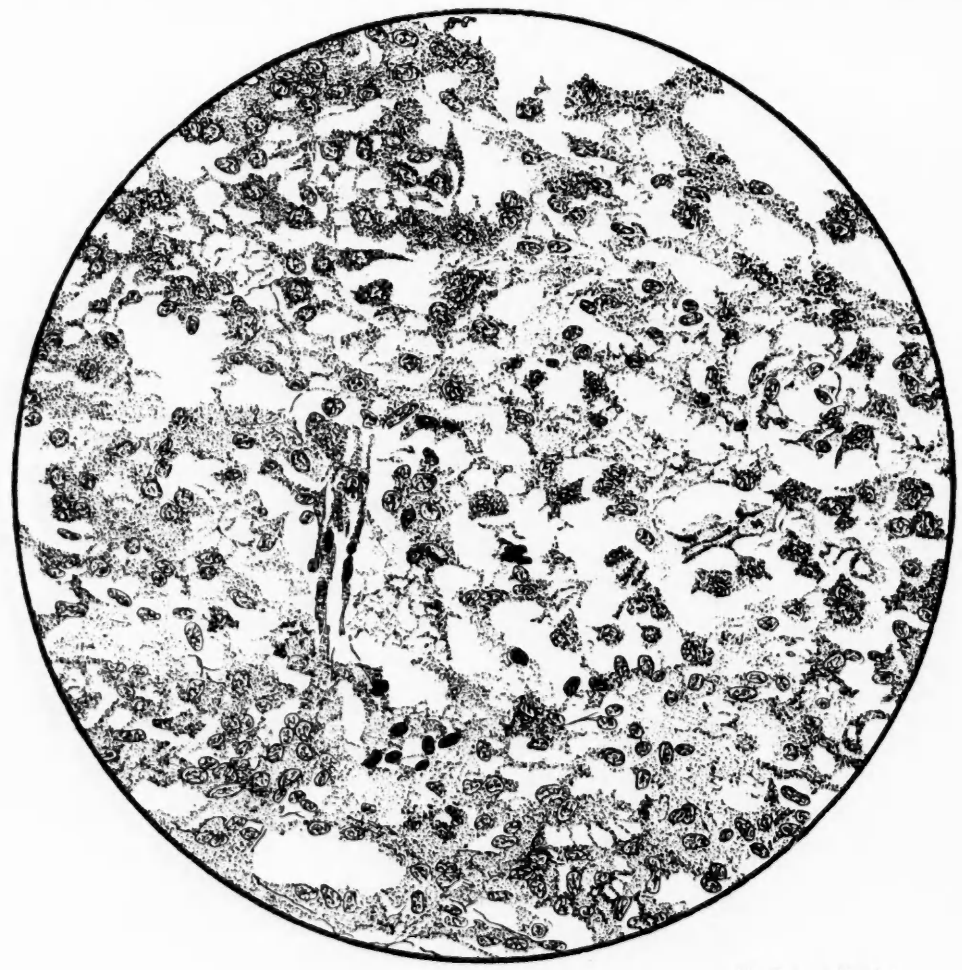


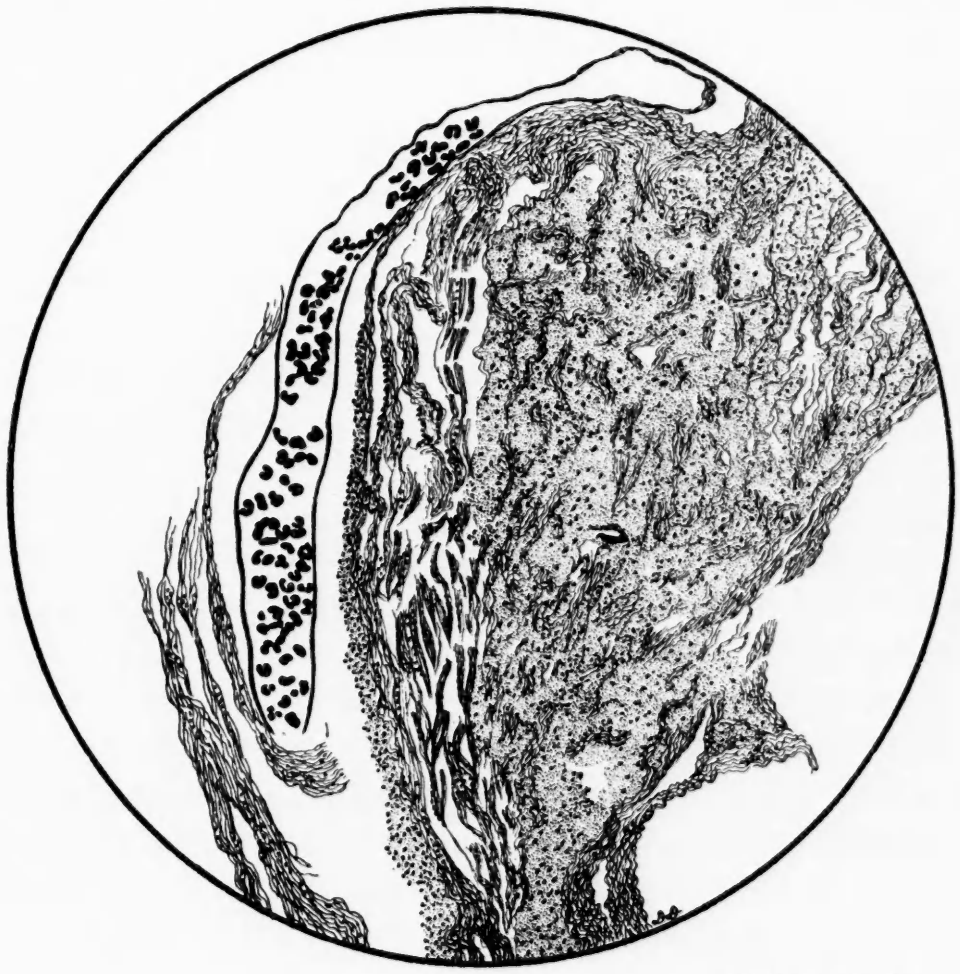
FIG. 5. Section in the stroma of the volvulus tumour. It shows a fibro-cellular stage, the cells being rounded or oval or spindle shaped. Giant cells and eosinophiles are also seen. × 275.



D. Dagnall, del.

FIG. 6. Section in the stroma near a group of worm sections. It shows degeneration of the endothelioid cells as detailed in the text. × 275.

FIG. 7. Junction of two capillaries in the areolar tissue outside the capsule of the volvulus tumour. It shows some perithelial thickening and a perivascular infiltration with plasma cells and a few lymphocytes. × 440.



D. Dagnall, del.

FIG. 8. A female worm to the left, piercing the fibrous capsule of the volvulus tumour obliquely, as detailed in text. × 55.

one of which showed seven nodules around the elbow and knee joints and varying in size from one to two inches; on puncture all the nodules gave negative results when examined for larvae and ova, while the skin examination proved positive for larvae in many regions. The other case was rather interesting. It presented four tumours around the right knee joint, three of which on puncture were negative for larvae and the fourth was positive, while the skin at many regions was heavily infected.

Sharp (1927) reiterated his observations of 1926 and stated that tumours were rather to be regarded as an incidental occurrence; common enough, it was true, but not invariable and in any case to be looked upon as late manifestations of the infection. Even the presence of nodules did not necessarily connote the existence of onchocerciasis for the worm might have long since been absorbed or cretified and no living organism be present. The one condition that was essential was the presence of embryos in the skin. The first sign of the disease was not a subcutaneous tumour, but the presence of embryos in and about the corium. The embryos were commonly found in the lymph glands of the groin and elsewhere, sometimes in enormous numbers. They were rarely found in the blood. He found them in the blood in 8 per cent. in a series of over 2,000 patients. The tumours were often intimately blended with the connective tissue of the surrounding fascia so that their removal could only be achieved with great difficulty. Yet they were almost always freely movable. They might be found at any part rich in lymphatics. They might occur near the knee, elbow or ankle joints, simulating juxta articular nodules so closely that in an endemic area one was bound to wonder from what the latter were derived if not from an extinct onchocerca tumour. He thus believed that juxta articular nodules were the aftermath of a fibrous tumour caused by *O. volvulus*, or other organism such as a spirochaete.

In size the tumours varied from a pin's head to that of a walnut. He came across two nodules of the size of a pin's head; he excised one and found embryos in it but could not detect the worm. He stated that if these were early tumours, then they might be formed by immature adults as they cannot accommodate an adult worm. So, therefore, it is probable that these tumours were formed by adolescent larvae which, in growing to maturity, gave rise to an

overgrowth of connective tissue and after producing batches of embryos from time to time, perhaps over a period of years, eventually paid their debt to nature and became the centre of a fibrous mass, which, at this stage, we recognise as a new disease entity : the juxta articular nodule.

Hoeppli (1927) examined a tumour of *O. volvulus* and histologically this proved to be formed of fibrous tissue rich in nuclei ; this fibrous tissue as a rule was closely attached to the numerous worm sections. In those parts he found specially slender pointed fibrous tissue cell nuclei, frequently arranged parallel to the cuticular surface of the worm. In the immediate surroundings of the parasite, the tissue as a rule was free from inflammatory infiltration. Between the cuticle and fibrous tissue in some cases there was a narrow cleft filled with solid exudate and numerous polymorphonuclear cells. The fibrous tissue was arranged in bundles. Its picture varied in different parts of the section : there were areas with the common slender nuclei especially surrounding the parasites, in other parts, being the majority, they were short, stout, ovoid nuclei and relatively little intermediate substance. Between these zones, there were frequently giant cells with nearly centrally placed nuclei. In several places the microscopical picture resembled that of giant celled sarcoma.

There were numerous microfilariae in the lymph clefts of the fibrous tissue, collections of microfilariae were found in the cavities filled with exudate. In the fibrous tissue there were several collections of eosinophile cells.

In the report of the Harvard Expedition to the African Republic of Liberia and the Belgian Congo (1930) it was stated that histological examination of the different volvulus tumours collected showed that they were all fibromata. In the centre sections of the adult parasites were commonly found usually embedded in the connective tissue stroma. Immediately around the parasites, there was some evidence of irritation caused by the parasite itself. A few polymorphs were scattered about and small round cells, and occasionally plasma cells and eosinophiles. Outside these areas the tumour was composed of fibrous connective tissue. Here the fibroblasts were few in number and the fibrils were not abundant. The tumour was composed particularly of collagen fibres forming wavy

bundles. In none of the tumours did the cells show marked evidence of mitotic division or numerous giant cells, so there was no evidence that the tumours were formed very rapidly. Sometimes there was no evidence of inflammatory changes about the sections of the adult parasites which were surrounded on all sides by wide bands of connective tissue in which collagen fibres predominated. In other instances there was a clear space about the section of the parasite which was partially surrounded by a thin layer of coagulated lymph in which fibrin and small round cells and polymorphs and a few plasma cells were visible. The surrounding connective tissue in such sections (taken apparently from tumours or areas of more rapid growth) was much richer in nuclei. These nuclei were more often round or oval, but sometimes were spindle-shaped. In some areas the appearance was somewhat suggestive of the condition observed in fibrosarcomata. Giant cells were present in some areas but were not abundant. In certain portions of the tumour, the eosinophiles were markedly increased, a condition not usually found in the immediate vicinity of the sections of the adult parasites. Sometimes small clumps of eosinophiles were found.

The microfilariae were found in large numbers in many but not in all parts of the tumours. In many areas in which they were prevalent the round or oval nuclei of the fibroblasts predominated in the surrounding tissue. Frequently there was a clear space or opening in the tissue about the organisms. Rarely were there accumulations of round cells or polymorphs about them. There seemed to be no doubt that the parasite was the inciting factor in the formation of the neoplasms.

Summary.

Thus the reader can gather from the above resumé of the pathological manifestations described by the various investigators the following points :—

(1) That *Onchocerca volvulus* embryos are found in the skin of patients who exhibit no tumours, and that the embryos in the tumours are very commonly found in the lymph clefts between the cellular elements.

(2) That the nodules vary in size from a pin's head to that of an apple.

(3) That the tumours show different microscopical pictures which may be summarised as follows :—

(*a*) There are tunnels around worm sections, the walls of which are composed mainly of cellular elements.

(*b*) The walls of other tunnels are made up of bundles of adult fibrous tissue with mature connective tissue cells in between the fibrils.

(*c*) That the stroma of the nodules is made up in certain areas of adult fibrous tissue, in other areas of merely cellular elements with a little or no fibrous stroma, while in still others it is fibrocellular resembling a fibrosarcoma, or the giant cells may predominate thus giving a picture similar to a giant celled sarcoma.

(4) That eosinophile cells are found in varying amounts in the worm tunnels or the stroma in the majority of the tumours.

(5) That the pathological manifestations exhibited by the disease differ widely in different countries ; thus, while the presence of the tumours declared itself in every endemic area, in the Konno district of Sierra Leone, elephantiasis, enlargement of the glands or skin lesions and eye symptoms are not conspicuous, while in the Congo elephantiasis and similar tumours and gland enlargements are very common and in Central America, in Guatemala, the eye lesions and erysipelas of the face are frequent.

In some districts various skin lesions are also attributed to onchocercal infections.

ONCHOCERCA GIBSONI AND SOME OTHER BOVINE SPECIES

Turning now to the pathological findings recorded in the case of *O. gibsoni* in cattle by some of the earlier and later workers on the subject, we find that Cleland and Johnston (1909) stated that the tumours were pale yellowish in tint like fibrous tissue and varied in size from that of a small marble to two inches or more in diameter. They were spherical when small and flattened or oval when large. On section towards the centre of the nodule were found the worms in an intricately coiled mass, embedded in a comparatively loose but resistant fibrous stroma with a varying quantity of serous or

sero sanguinous fluid. This fibrous tissue is tunnelled by canals occupied by the parasite.

The central worm mass varied very little in size in comparison with that of the whole tumour. This central mass was completely enclosed in a delicate capsule which could be shelled entirely from the dense fibrous tissue forming the external covering. This outer covering was of very dense white fibrous tissue, much denser than that between the coils of the worm and varying in thickness according to the size of the tumour.

Microscopically sections of the outer wall showed that it consisted of dense fibrous tissue containing a variable number of blood vessels and cells varying in number in different parts. In some places were scattered leucocytes containing numerous eosinophile granules. These may be dispersed singly or form columns between the fibrous tissue or be collected into considerable masses in a little fibrous stroma. Such cells constituted by far the majority of those met with in sections of the wall. Ordinary polymorphonuclear cells were not noticed. In addition to these cells were a varying number of connective tissue cells and also in the fibrous stroma itself, branching cells with basophile granules, the mast cells of the tissue. They found instances in which the worm, dead in the nodule, had become in part calcified as evidenced by the presence of gritty material effervescing with acid which was collected in small masses resembling the coils of the worm. In other cases, where the worm had died, the parasite was soft and becoming yellowish, and the surrounding tissue was also degenerated.

Gilruth and Sweet (1911) stated that the nodules were practically confined to two distinct and separate situations, viz. :—(1) The brisket region, and (2) the external surface of the hind limbs. The size of the nodules varied from a split pea to that of a mandarine orange. On section the tumour was seen to be composed of a very dense fibrous wall which varied in thickness in different nodules and to enclose the tightly coiled mass of worms in tunnels lying bathed in a small quantity of opalescent fluid. Calcareous degeneration was not uncommon and occasionally a purulent form of degeneration might be seen in which the fragments of the dead parasite lay free. Often these degenerations attacked only one portion of the worm area, the other still containing undegenerated parasites with living

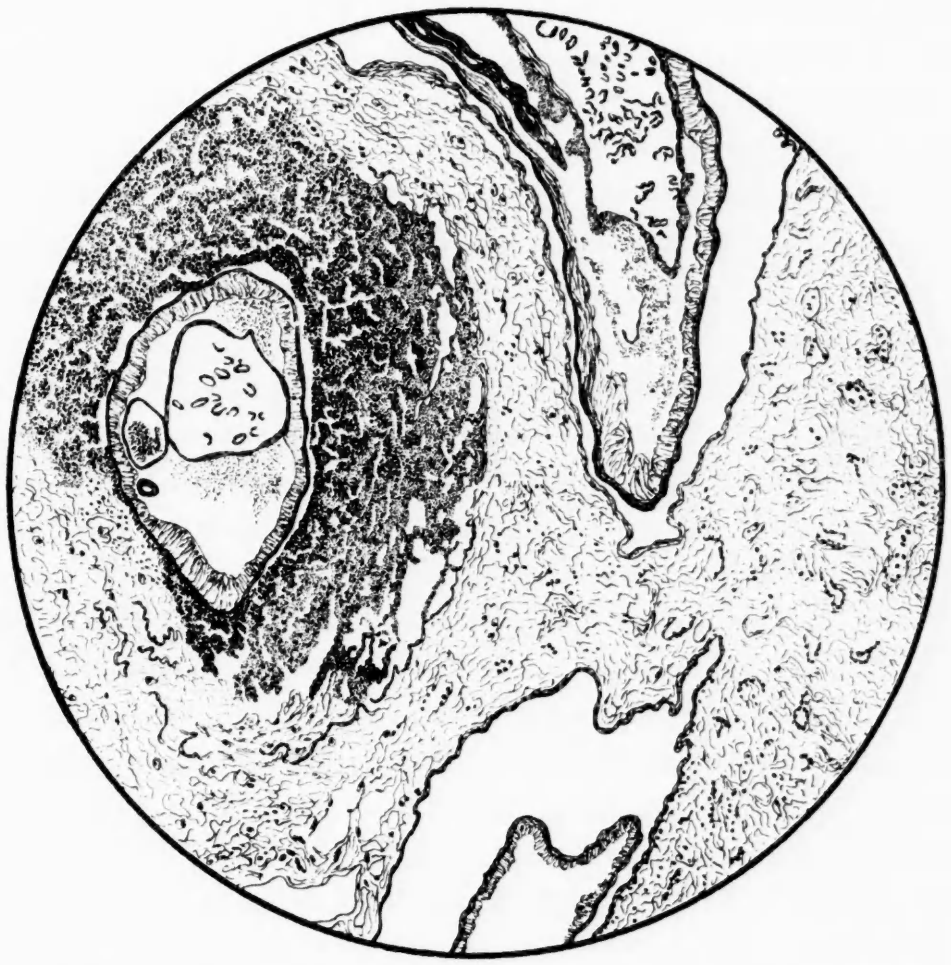


FIG. 9. Shows three worm sections in the gibsoni tumour; the upper two are surrounded by adult fibroid tissue and the lower is surrounded by a thick layer of reactionary cells, as detailed in the text under (1) and (2). × 55.

FIG. 10. Section in the middle part of the gibsoni capsule, showing embryos lying in lymph clefts and surrounded by a fibro-cellular tissue. × 440.

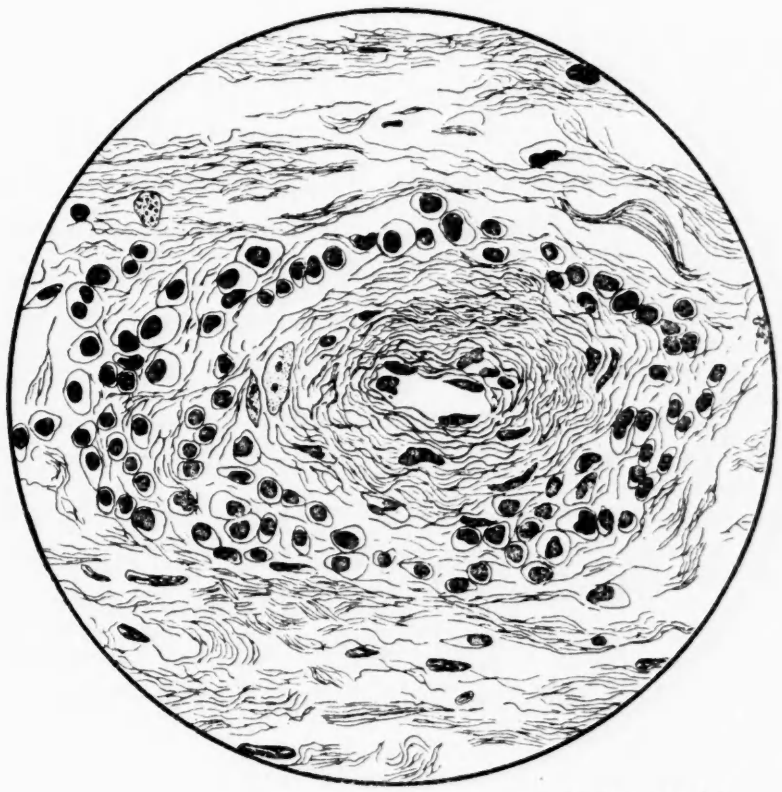


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FIG. 11. A blood vessel in the gibsoni capsule, showing marked thickening and a perivascular infiltration with lymphocytes and plasma cells. × 440.

larvae. These degenerations appeared solely to affect the parasites and the tissue between the coils. Degenerated nodules were more common in older animals.

Microscopically, the capsule was made of dense fibrous tissue with many nuclei and processes therefrom passed inwards and branched and anastomosed with each other to form the tunnels in which the worms lay.

In the smallest nodules the fibrous capsule was very thin, and the branches that went to form the tunnels were very delicate. A notable feature of the capsule especially in larger tumours was the great thickness of the walls of the arteries compared with the very small size of their lumina. This thickening was due to a periarteritis and was often accompanied by endarteritis and not infrequently by obliteration of the lumen. In thinner capsules, particularly those of small nodules, the thickening of the arterial wall was not marked.

In those nodules composed of much fibrous tissue, numerous larvae could be seen traversing the tissue and often aggregating in the vicinity of the blood vessels, whereas in those with very thin capsules migrating larvae were seldom if ever seen.

The fibrous bands forming the walls of the tunnels varied much in thickness and density, frequently they were only represented by a few delicate strands ; at other times, especially towards the surface of the worm area, the breadth of these bands exceeded the diameter of the tunnels.

In the deeper portions of the worm area especially around the heads of the parasites, the tissue was much looser, the nuclei were more numerous and there was generally greater or less infiltration by leucocytes and especially eosinophile cells which were often massed at the edge of the tunnel wall. The leucocytes underwent degeneration in certain areas as shown by nuclear disintegration and indefinite staining reaction.

These authors again (1912) kept two infected cows under observation for six to eight months and killed them. They found a fair number of degenerate nodules in each and noticed a marked diminution in the size of these nodules during the period of observation. They stated that among the large number of nodules they formerly examined, the female worms were either in a *stage of complete development liberating living larvae or the nodules contained*

degenerate parasites ; i.e., no immature parasite had ever been found in a nodule.

They found in a very old bullock a few small circumscribed circular or oval dense fibrous masses about half to three-quarters of an inch long and adhering firmly to the muscular-fascia. Section showed a calcareous centre.

Sweet (1914) stated that the nodules from the buffalo differed in external appearance from those found in cattle, the capsule of the free nodules being at times much thicker than in cattle and the fibrous tissue much less compact in the outer part of the capsule, though at the same time it was very tough and resistant to cutting or tearing. At other times a continuous capsule wall was absent and only a trifling amount of fibrous tissue was present.

The trabeculae forming the walls of the worm tunnels had a considerable quantity of fibrous tissue. She observed that these nests showed more tendency to calcify quickly in the buffalo than in cattle. She also noticed that considerable caseation was present in several nodules due to degeneration of the worms or parts of them. A large proportion of nodules of *Onchocerca indica* seen by her were calcified or caseated due to the fact that only aged cattle were killed in the country from which she got them. She noticed that the nodules of *O. indica* in cattle were smaller and had much thinner capsules than *O. gibsoni*. The whole tissue of the nodule was permeated by larvae even in cases where mature worms showed no larvae in the genital tubes. Also larvae were found on the periphery of the nodule and sometimes thickly in the loose connective tissue on the surface of the nodule.

Cleland (1914) found embryos of *O. gibsoni* in a thickened area of the skin of the belly of a calf and on post-mortem examination no nodules were found near that area. On serial sections the embryos were found at various depths below the epithelium. He found an onchocerca about eleven and a half inches long in the loose tissue of the back part of the knee joint in a bull. This worm was not encapsulated though a small fibrous nodule of the size of a split pea was found attached to a part of it. The worm itself showed active movements. In one instance he found a group of three females containing living embryos, and two males loosely coiled near the hip joint. These worms were not surrounded by

any fibrous tissue which meant that the adult worms occurred unencapsulated and moved freely in the tissues depositing their embryos, and that encapsulation may only be an accident. A portion of another worm was found embedded in a lymphatic gland quite near to the above group. The portion of the worm in the gland was lying in a fibrous track surrounded by lymphocytes and also giant cells which were an evidence that it had been caught in the gland for a long time. He stated that this group of adult worms was migrating in the tissues, but the arrest of a part of one of them in the lymphatic gland had led, by its entanglement with its fellows, to the arrest of them all and stoppage of their free movement. These worms were adult as the females had living and actively motile embryos in them and even projecting from the vagina. This proved the fact that the worms had reached maturity although they were entirely unencapsulated. This raised the question as to whether encapsulation was or was not a normal process in the life-history of *O. gibsoni*. He thinks it highly likely that *O. gibsoni* like *Filaria loa* normally wanders through the subcutaneous tissues, depositing from time to time during its progress living embryos in the tissues through which it migrates. Such deposits of embryos would account for their presence in the skin and especially for the instance in which a number was found in a small swelling in the belly of a calf far removed from encapsulated worms. It is probable that the embryos so liberated, by means of toxic products, cause some amount of swelling and this may eventually lead to a certain degree of fibrosis. When for some reason or other the adult worm is arrested the embryos which still continue to be liberated produce a reaction in the surrounding tissues, so that the worm eventually becomes encapsulated in a dense fibrous mass which increases in size as time goes on. Probably in the early stages of such encapsulation the embryos can escape but eventually the fibrosis becomes so dense that none can find their way through and the worm dies from cutting off its food supply.

In one of his drawings he showed a blood vessel with two embryos lying actually in the thickness of the wall of the vessel.

Chrétein (1920) found in the ox in Madagascar a nematode worm, often present in abundance in almost all the adult animals under his observation. Its occurrence was so common that in his

opinion the parasite could be found in all cattle submitted to a careful examination.

The parasites were situated in the connective tissue fascia adjacent to the ligaments and tendons, but they were not actually found within these structures. The lesions produced might be seen particularly within the loose connective tissue lying between the various layers of the ligamentum nuchae and especially in the uppermost layers of the ligament, often near its attachment to the superior spinous processes of the first dorsal vertebrae. The parasites and lesions were, however, also quite numerous in the fascia surrounding the articular ligaments especially those of the knee and hock joints, and in the fascia of the tendons of the limbs.

When the parasite occurred singly, as it did fairly commonly in the limb tendons, but rarely in the cervical ligaments, it was not easy to demonstrate, and he had to dissect out carefully the fascia surrounding the tendon or even cut thin slices parallel to the tendon and examine them by transmitted light. A worm about as thick as a thin thread was observed embedded in the connective tissue in which it was always more or less loosely tangled and almost impossible to follow along its whole length. Single specimens did not appear to provoke any naked eye lesions in the surrounding tissue at this stage. By means of a fine needle the worm could be easily extracted from the tissue in the form of a fine lustrous thread, often up to 10-12 cm. in length which assumed a corkscrew-shaped disposition. This did not represent the whole parasite as the body became invariably ruptured before the extremity was reached.

If, however, instead of single specimens, several worms were present in the same location as was usually the case when the parasites infect the ligamentum nuchae, the fascia became slightly thickened and opaque. The thickening was readily detected on manipulation and the fascia was always closely adherent to the tendon at the part. These lesions were distributed in irregular zones of considerable size and of a slightly brownish tint, which were again often surrounded by oedematous areas of fairly considerable extent. On close examination a certain number of parasites might be detected in the central zone of these lesions in the form of more or less abundant closely packed and entangled masses of worms. These produced a distinct inflammatory reaction in the invaded

tissue. They could be again readily extracted partially, but they appeared to have become less supple.

Other lesions presented the aspect of a distinct chronic inflammation, but the parasites within these were more compact and it was difficult to extract pieces of more than 1 cm. in length, and the worm had become brownish and brittle.

In the ligamentum nuchae and sometimes in the limbs greenish abscesses from the size of a pin's head to that of a pea were often found. In these situations also it was common to find a number of irregular nodules generally flattened, measuring up to 1 cm. in diameter, and of a dull white in colour. These were filled with a dessicated caseous material.

The above appearances were said to represent successive stages in the development of the parasite.

Histological examination confirmed the author's observations with regard to the situation of the parasites, i.e., in the fascia surrounding the tendons and ligaments and not within these structures.

To commence with there appeared to be no appreciable re-action, the worm simply burrowed a cavity in the tissue which did not re-act. Inflammatory phenomena were not manifested until after a certain lapse of time. Around the parasite a centre of subacute inflammation was formed similar to that seen in the majority of worm nodules and characterized by the formation of a mass of concentrically disposed epithelioid cells. Fibrous tissue becomes formed without and the adjacent fascia becomes denser and sclerosed. The lesion remained at this stage while the parasite was still alive, but after its death the inflammatory phenomena became more marked and large nodules were formed with caseous centres impregnated with alkaline lime salts which took the haematein stain deeply. The walls of these nodules were made up of an epithelioid zone and a fibrous zone, the thickness of which depended on the age of the lesion. The reaction extended to the connective tissue situated between the spirals of the worm which became converted into a sclerosed tissue infiltrated with numerous eosinophiles. Finally the lesion became the centre of firm connective tissue traversed by a devious tunnel full of caseous substance which replaced the dead parasite.

Microscopic examination of the parasite showed that it was a nematode with a thick striated cuticle showing corkscrew-like

thickenings, often interrupted towards the sides and thus belonging to the genus *Onchocerca*. The writer stated that onchocerciasis in cattle in Madagascar was reported for the first time by Piettre in 1912 in a leg of beef imported into Paris. Piettre believed that the parasite was *Onchocerca bovis* which he discovered the same year in French cattle. The author did not agree with the view that the parasite he encountered belonged to this species; perhaps they belonged to several species.

Heydon (1927) recorded the presence of the larvae of *O. gibsoni* in the superficial layers of the dermis in Queensland cattle having nodules of *O. gibsoni* in the brisket, the number of embryos varying from 15 to 40 per square millimetre of skin.

De Kock (1928) in speaking about onchocerciasis in the cattle of South Rhodesia, maintained that the nodules occurred only on the brisket in a **V**-shaped area with the point on the crest of the sternum and the limbs extending caudally to a point two inches past the xyphoid cartilege. No nodules were observed on the external surface of the hind limbs as in Australian cattle. The incidence of the disease was much less and also the number of nodules in an infected animal; the nodules usually occurr singly and seldom in twos or threes. Macroscopically they were divisible into two groups according to their position in the subcutaneous connective tissue: (*a*) very superficially placed nodules that could hardly be said to be situated in the subcutaneous tissue, appearing as flat ovoid tumours on the surface like buttons; (*b*) nodules which appeared as swellings in the subcutaneous tissues revealing their presence by a slight bulging of the connective tissue. The nodules were very tough and fibrous. On section the superficial button-like nodules showed a thin fibrous capsule enclosing a well-defined irregular nucleus or 'worm-nest' proper. This capsule measured up to 2 mm., but in the majority only 1 mm. in thickness. The deep-seated tumours were elastic on the outside, but a hard firm centre could be felt, thus giving the impression that the firmness increased from without inwards. On section the capsule was noticed to be much thicker enclosing a well-defined worm-nest. On measurement it was found that although the deep-seated tumours were much larger than the superficial ones, they did not enclose a larger nucleus; e.g., a tumour 3·9 cms. in diameter had a worm area of only 0·9 cm. in diameter,

while on one side the capsule was 1·3 cm. and on the other 1·7 cm. thick. Others again showed a thinner capsule, 5·0 cm. thick and the worm area of practically the same size. Thus it would be seen that the thickness of the fibrous capsule largely depended on the situation and that the size of the worm-nest had no relation to the thickness of the capsule around.

The worm-nest, although well defined, was difficult to enucleate from the capsule owing to small irregular trabeculae passing from the capsule into it and others passing from it to the capsule. On section the worm-nest showed a greyish-white matrix in which were numerous tunnels of pin-point size, cut through transversely, diagonally or longitudinally. These tunnels and furrows were occupied by the very much contorted onchocerca surrounded by the matrix. According to Gilruth and Sweet the female worm attains a length of 140 cm., and nodules appear to contain generally one female only and less commonly one male and one female. Very often the worm centres were calcified, especially in old animals. In the cut surface then, instead of tunnels and furrows, the spaces were filled with a yellowish-white gritty substance which came off as small solid cylinders on scraping. In older tumours nothing could be distinguished as calcification was too far advanced. Microscopically all the tumours were formed on the same general basis. In the capsule the line of division from the normal connective tissue was distinct on the outside. Inwards there was a steady increase of spindle-shaped fibroblasts up to a line close to the margin of the worm nucleus where they were very densely packed.

Blood vessels in this area were fairly numerous with their walls thickened and in some instances with their lumina obliterated. Round the blood vessels, looking like scattered islands, was an infiltration of polymorphonuclear leucocytes and eosinophiles in preponderance. There were also round cells present.

Towards the centre amongst the densely packed fibroblasts were scattered eosinophiles increasing in number towards the centre, also neutriphiles and round cells. The zone of closely packed fibroblasts was rather well defined on the inside, breaking off sharply with a few scattered fibroblasts still extending centre-wards. From this zone the polymorphs and round cells increased rapidly in number and this marked the line of demarcation between the capsule and the worm-nest.

The worm nucleus, from the line of demarcation, except for a few spindle-shaped fibroblasts, was completely built up of polymorphonuclear leucocytes of which a fairly large number were eosinophiles and other round cells forming the matrix in which the worm lay coiled up. Near the border there was practically no loss of structure in the cells, but extending a short distance inwards the cells showed necrobiosis and the matrix was finally built up completely of such altered cells with a few fibroblasts.

The matrix showed the numerous openings described above in which the worms lay.

Onchocerca in section. The body wall showed indistinct striation. Enclosed in the body cavity appeared the intestinal tract to one side and a double uterus containing eggs. In different sections of the uteri various stages of development of the embryo could be made out. The complete development of the embryo could be studied in one nodule, from a cell with a single nucleus, to the morula stage with numerous nuclei and to small larvae lying coiled up in the uteri. Between the worm and the matrix was a thin layer of homogeneous substance, viz., the fluid in which the worm lay.

Summary.

From the above resumé of the pathological findings by the various investigators in *O. gibsoni* infections, the following conclusions could be drawn.

1. That the skin may exhibit infection with larvae in places far removed from the tumours.

2. That the adult male and female worms lead a free unencysted life wandering in the tissues and discharging embryos.

3. That portions of adult worms are found in an encysted condition ; the rest of the bodies of these worms are still free in the tissues and exhibiting active movement.

4. That the capsule of the tumours exhibits much variation in thickness, while the worm area shows little variation in size and that the size of the large tumours is contributed to greatly by the much thickened capsules, the blood vessels of which show marked thickening or even complete obliteration.

5. That the tunnel walls surrounding the worms and the stroma in between these and the capsule of the nodules show varying degrees of cellularity or fibrosis.

6. That the eosinophile cells formed a conspicuous element in the majority of these tumours.

7. That Secondary degenerative changes and calcification in the worms in the tumours were commonly met with.

EXAMINATION OF MATERIAL

Professor Blacklock handed over to me an *Onchocerca volvulus* tumour about 1 cm. in diameter with a small accessory tumour about 2 mm. in diameter attached to it on one side. This was removed by him from a patient in Sierra Leone. He also gave me a nodule of *Onchocerca gibsoni* removed from the brisket of an infected animal killed in Australia. This tumour was about the size of a hazel nut and oval in shape. He asked me to make a full study of the pathology of these tumours and to find out how far the histological picture represented by them agree or differ from each other. I must here make it clear that in order to make a detailed study and full comparison, a large collection of tumours of different ages and sizes and removed from a large number of persons or cattle would be necessary for examination, in order to be able to follow out all the histological manifestations and the different pathological stages or phases that may present themselves in these growths.

The tumours were passed through formalin, alcohols, and embedded in paraffin, and sections cut and stained, with haematoxylin and eosin in the usual routine way.

Microscopically the *O. volvulus* tumour is found to be surrounded by a relatively thin capsule of adult connective tissue, brown in colour in some parts and made up of dense compact bundles of fibrils, amongst which are a number of mature connective tissue cells or fibroblasts with long, slender, spindle-shaped nuclei. In some parts, this fibrous capsule is fairly vascular, especially in its outer layer, the blood vessels looking quite normal and not thickened. No microfilariae were seen in the capsule.

Outside the capsule is a layer of loose areolar tissue which is also fairly vascular. In the part of this areolar tissue which lies in the angle between the main tumour and the accessory small nodule are seen islands of plasma cells with some lymphocytes amongst them and a few giant cells lying in close relation to the

blood vessels in this area, the walls of which show a slight thickening. In this same area of areolar tissue, in one of the sections was seen a thick coiled embryo lying just outside a blood vessel and another similar one a little distance away. Other thick stout coiled embryos were found in the same situation in another section. These embryos look distinctly thicker than the free ones which are seen in the tumour spaces. Their thickness is like that of the coiled embryos lying inside the egg-membrane in the uterus of the worm. The writer is inclined to believe that these thick embryos were dislodged from one of the cut sections of the female worm lying in the accessory nodule near by in the process of sectioning.

The small accessory nodule is found adherent to the capsule of the main tumour on one side. It has an incompletely formed capsule on its far end, this capsule is formed of immature fibrous tissue with numerous cells, round or oval in shape and the fibrils are delicate. In this part of the capsule are seen some microfilariae.

In the interior of this nodule are seen three transversely cut worm sections lying in thin canals of immature fibrous tissue composed of numerous cells with round or oval nuclei and delicate fibrils. The stroma of this nodule is made up of endothelioid cells with rounded or oval nuclei and many giant cells and some plasma cells and eosinophile cells scattered amongst these and a very little network of delicate connective tissue fibrils running in bands between the cell masses. Amongst these cells are seen some microfilariae lying free.

Returning to the main tumour, it is found to be traversed by tunnels containing sections of the worms cut, some transversely and others longitudinally. Some of the tunnels lie immediately under the fibrous capsule of the tumour.

The histological picture of these tunnels and of the intervening tissue varied very much in different areas.

1. In some areas the worm section is seen surrounded by a thick layer of endothelioid cells with rounded or oval nuclei and little if any stroma of fibrous tissue. Scattered amongst these endothelioid cells, and especially numerous immediately outside the cuticle of the worm, are seen large giant cells with finely granular eosinophilic cytoplasm and spongy nuclei not taking the stain deeply. Those giant cells lying in apposition to the worm are occasionally flattened

and elongated, forming long syncitial masses of protoplasm and nuclei.

Amongst the endothelioid cells are occasionally seen a few polymorphonuclear cells, eosinophile cells, lymphocytes and plasma cells. Sometimes little collections of polymorphs and eosinophiles appear amongst the giant cells outside the cuticle. The blood vessels are thin-walled.

2. Worm sections surrounded by a cellular layer similar to (1) especially immediately around the worm but with fibrosis occurring at the periphery of these worm tunnels. The endothelioid cells diminish in number and fibroblasts with elongated nuclei and fine fibrils replace them.

3. Worm sections surrounded by immature cellular fibrous tissue, i.e., there is a delicate network of fibrils with numerous cells between them, some are of the round-celled type and others of the fibroblast type with elongated nuclei. Giant cells and eosinophile cells are also seen amongst these cellular elements.

4. Other sections of the worms are lying in tunnels, the walls of which are made up of whorly bundles of adult fibrous tissue with a relatively small number of connective tissue cells with elongated nuclei in between the fibrils. These are older tunnels than the others. In such tunnel walls no giant cells or eosinophile cells are seen either in the immediate vicinity of the cuticle of the worm or amongst the bundles of fibrils forming the tunnel wall. The fibrous wall of these tunnels is thin and is much smaller in thickness than the diameter of the tunnel itself. These old fibrous tunnels occur especially under the capsule of the tumour.

5. The pictures of the matrix of the tumour mass or the intervening tissue between the worm tunnels exhibits some variation in different areas, viz. :—

(*a*) In some places it is made up of masses of endothelioid cells with rounded or oval spongy nuclei not taking the stain intensely. Amongst these cell masses are occasionally seen giant cells. Very few delicate connective tissue fibrils are seen passing between these cell masses. A few plasma cells and occasionally eosinophile cells are also seen.

(*b*) At other places the picture is that of very cellular immature fibrous tissue. Bundles of fibrils are seen running in a wavy

manner and amongst these are numerous cells, some being rounded with round or oval nuclei, and others are elongated with spindle-shaped nuclei. Plasma cells are occasionally seen and eosinophile cells are also present.

(*c*) Other fields show a picture very similar to the above one with the addition of a fair number of giant cells; the picture resembling very much that presented by Hodgkin's disease. The giant cells here differ in many respects from those described above which occur immediately outside the cuticle of the worms or in the tunnel wall in (1). They here appear small and more compact and take various shapes, thus becoming flattened, or radiating in close apposition to the cellular and fibrous elements around. This is due to the pressure of the growing fibrous tissue around them and also to the counter pressure of the capsule of the tumours. Their protoplasm is not so eosinophilic as the above-mentioned giant cells and their nuclei are pressed together into one or more solid masses and take the stain deeply.

(*d*) Areas made up of adult fibrous tissue bundles with mature connective tissue cells or fibroblasts amongst them. These areas occur especially near the periphery of the tumour.

(*e*) In a few places near the worm sections, especially in areas where these sections are numerous and lying nearly side by side, the endothelioid cells have undergone a good deal of necrosis and degeneration; their outlines are lost, the protoplasm is disintegrating and the nuclei which take the stain faintly are lying nearly free amongst the protoplasmic remains and an irregular fibrinous network. This is possibly due to toxin liberated from the numerous coils of the worms around, poisoning these cells whose function was to lay the foundation for a process of fibrosis around these coils, but were overwhelmed before there was any chance for the fibrosis to start.

The matrix of the tumour is fairly vascular, the vessels are either thin-walled or normal but not thickened.

6. The sections of the worm in the tunnels show the gut and the uterine tubes containing in certain fields non-segmented ova, in others ova at the morula stage or ova containing coiled embryos, or

the embryos are seen lying free and flat in the uterus. The worms in this tumour must have been adult mature parasites. Sections of male worms are also seen in some tunnels.

It was noted that one of the sections of this tumour showed a female worm cut longitudinally with a part of it lying outside the capsule of the tumour and the rest piercing this fibrous capsule obliquely and lying in a tunnel surrounded by the dense fibrous bundles of the capsule. The tunnel wall is made up of the dense fibrous tissue of the capsule externally and next to the worm is lined by a thin layer of endothelioid cells with some giant cells, eosinophiles, a few polymorphs and plasma cells.

PATHOLOGY OF *O. GIBSONI* TUMOUR

In the sections one's attention is drawn to the great thickness of the capsule of the tumour compared with the diameter of the worm area proper. This is even apparent to the naked eye if one holds the section against the light. On one side the thickness of the capsule is nearly equal to the diameter of the worm area proper. One also sees a blue line running round the worm area between it and the capsule.

Microscopically the capsule is made up of fasciculated bundles of very fine connective tissue fibrils, which run round the circumference of the worm area and intercommunicate with each other, leaving a multitude of empty spaces between the bundles. This picture may have been due to an oedematous condition during life in the nodule. The fibrous tissue is fairly cellular, the cells being mostly of the adult connective tissue type with elongated spindle-shaped nuclei; they run parallel to and among the fibrils. The inner layer of the capsule which lies next to the worm area is more cellular than the rest of the capsule, the majority of the cells being of the adult connective tissue type mentioned above. Other cells with large round or oval nuclei and also a few giant cells and eosinophile cells are seen amongst the bundles of fibres in this inner zone of the capsule.

A large proportion of the connective tissue fibrils of the capsule in its outer zone, have undergone hyaline transformation. In the capsule, especially in the outer layers, are seen large and small

deposits of lymphocytes: these either occur in the form of islands or as elongated chains running along the direction of the fibres.

The blood vessels of the capsule, in its outer zone, are fairly numerous and their walls much thickened, due to both peri and endarteritis; the lumina are much narrowed or even completely obliterated. Large deposits of lymphocytes with some plasma cells are seen around the majority of these vessels.

Throughout the thickness of the capsule and especially numerous in its inner cellular zone are seen many microfilariae, either straight or curved, and numbers of them lie in the clefts or lymph spaces between the fibrous fasciculi.

The worm area proper in this tumour is small and contains a small number of worm tunnels enclosing the worm sections, and the rest is made up of fibroid tissue between and around the tunnels, making the stroma of the worm nucleus. This fibroid tissue is of the hyaline type, resembling the scar tissue left after the organization of tubercular deposits. Scattered in this fibroid tissue are seen many eosinophile cells, giant cells, fibroblasts with elongated nuclei and also polymorphonuclear cells. Plasma cells are also present. There are numerous empty clefts and also dilated lymph spaces due to, perhaps, some oedema during life. This fibroid tissue is also poorly vascular and the blood vessels are not a prominent element as in the capsule.

Very few microfilariae are found here, which makes a prominent contrast with the number in the capsule.

The structure of the tunnels in which the worm sections lie, exhibits certain definite variations.

1. There are tunnels, the walls of which are made up of the same fibroid hyaline tissue described above in the stroma, with similar cellular deposits, the tunnel wall merging imperceptibly into the stroma of the worm area. These are old tunnels as there is no trace of any recent reaction in their walls, the whole process is completed. Lying free in the tunnels and sometimes adherent to the cuticle of the worms are sometimes seen clumps of polymorphs and eosinophile cells and a few plasma cells. The innermost layer of fibroid tissue of the tunnel walls here, which lies next to the cuticle of the worm, is more compact than the rest of the stroma, thus making a sort of a ridge.

2. There are worm sections surrounded by a thick zone of cells, many of which are broken up. These cells are eosinophile cells, polymorphonuclear cells, lymphocytes and plasma cells. In the outer layer of this cellular zone are seen plenty of endothelioid cells, mostly broken down and their nuclei fragmented. Outside this is seen the hyaline fibroid tissue creeping inwards and continuous on the outside with the hyaline fibrous tissue of the stroma.

3. Worm sections are present, surrounded by a zone of broken and creeping hyaline fibrous tissue coming inwards from and continuous with the stroma outside it. Entangled in the meshes of this encroaching fibroid tissue are seen fragments of nuclei and a few eosinophile cells, polymorphonuclear cells, and plasma cells. Immediately outside this zone are seen numerous giant cells in the stroma. Lying in the tunnels are seen sections of male and female adult and gravid worms with the uteri containing mature embryos and also eggs in different stages of development.

THE GENESIS OF THE TUMOURS IN ONCHOCERCAL DISEASE

Labadie-Lagrave and Deguy, as previously mentioned, examined a volvulus tumour removed from a French soldier in which they found the immature female worm lying free in a dilated and inflammatory changed lymphatic vessel, surrounded by a fibrinous exudate containing numerous leucocytes and phagocytes. Brumpt accepted this view and stated that an inflammation occurs in the lymphatic vessel in which the worms lie, the resulting exudate surrounding the worms being organised into fibrous tissue. In older tumours a lymphatic vessel could no longer be recognised as a point of origin of the tumour formation. Fülleborn did not feel justified in coming to a decision on this point. Cleland proved in the case of *O. gibsoni* that the adult worms occur unencapsulated and wander in the tissues liberating their embryos. When for some reason or other the adult worm becomes arrested, the embryos which still continue to be liberated, produce a reaction in the surrounding tissues, so that eventually the worm becomes capsulated in a dense fibrous mass which increases in size as time goes on. Probably in the early stages

of such encapsulation the embryos can escape, but eventually the fibrosis becomes so dense that none can find their way through and the worms die, from the cutting off of the food supply.

Sharp (1927) found two nodules the size of a pin's head. On excision one was found to contain embryos but the worm itself was not detected. He commented that if these were early tumours, they might be formed by immature adults, as they could not accommodate an adult worm. He is of opinion that the tumours are probably formed by adolescent larvae, which, in growing to maturity, give rise to an overgrowth of connective tissue and after producing batches of embryos from time to time, perhaps over a period of years, eventually become the centre of a fibrous mass which is the juxta-articular nodule.

The above is a resumé of the origin of tumour formation found in the literature. The theory that the adult worms live in the lymphatic vessels and ultimately form tumours inside these can be abandoned, for if they do so they would behave in exactly the same way as the adult *W. bancrofti* worms and the presence of the *O. volvulus* embryos in the blood would then be the rule rather than, as it is in fact, the exception. Further, it is quite easy for a lymphatic vessel to accommodate inside it a worm 3 to 4 inches long, as *W. bancrofti*, but it is difficult to understand how a worm 50 to 70 cm. in length (Baylis), like *O. volvulus*, can accommodate itself inside a lymphatic vessel. Further, against Sharp's contention that the tumours are started by adolescent larvae reference may be made to the observations of Gilruth and Sweet (1912) on *O. gibsoni* tumours. They stated that among the large number of nodules they examined the female worms were either in a stage of complete development liberating living larvae or the nodules contained degenerate parasites, i.e., no immature parasite had ever been found in a nodule.

Moreover, the writer believes that the young adolescent worms by comparison with the young members of any race including mankind, must be endowed with their fullest powers of activity to try to overcome any obstacle that hinders or arrests that active life. When they become adults and the female is fecundated, especially when the latter produces larvae for a very long time, then the free worms begin to become sluggish and seek rest and shelter elsewhere. The writer thus agrees with Gilruth and Sweet that it is the adult worms

and not the young ones that ultimately become the nucleus for a tumour formation.

The earlier workers used to think that *Onchocerca volvulus* and *O. gibsoni* were associated with the presence of tumours and nothing else, and onchocerciasis was recognised as a condition in which these tumours were found. Ouzilleau, Laigret and Lefrou (1921) noticed that subjects infected with *O. volvulus* always had microfilariae in the dermis. Blacklock (1926), in Sierra Leone, out of 68 examined for nodules and larvae found 44 per cent. with larvae in the skin but only 40 per cent. with nodules. Sharp (1926), at Kaduna in Northern Nigeria, reported the skin infection among 100 prisoners as 55 per cent., while the presence of tumours was only noted in 30 per cent. of these. Cleland (1914) found embryos in the case of *O. gibsoni* in a thickened area of the skin of the belly of an animal and on post-mortem examination, no nodules were found near the area and on serial sections ; the embryos were found at various depths below the epithelium. Further, in one instance, he found a group of three females containing living embryos and two males loosely coiled near the hip joint. These worms were not surrounded by any fibrous tissue, which means that the adult worms occurred unencapsulated and moved freely in the tissues, depositing their embryos. Cleland, therefore, is of opinion that encapsulation of the worms is an accident, and it is most likely that *O. gibsoni*, like *Filaria loa*, normally wanders through the subcutaneous tissues depositing embryos in the tissues through which it migrates.

Heydon (1927) also recorded the presence of the larvae of *O. gibsoni* in large numbers in the superficial layers of the dermis in cattle having the nodules in the brisket.

From the above data, one is able to judge definitely that both the adult *O. volvulus* and *O. gibsoni* worms lead a free unencysted life and wander in the tissues depositing their embryos from time to time in these as they move about, and that it is not necessary that tumours should be present in onchocercal infections.

The second point that confronts us is how these tumours are formed. It appears improbable that any of the above mechanisms fully explains the genesis of the tumours and their relationship to the worm. We have seen that the adult worms may lead a free life in the tissues and deposit their embryos as they move about. This free wandering

life may continue for a long time, possibly years. That they actually move about can be inferred from their anatomy as the cuticle is rough and marked by ridges whose function must be to help in the forward movement. Before the worms become encysted in the tumours and while they are wandering about in the tissues, they must be in an uncoiled state. After a long or short time of free wandering life in the tissues, and for some as yet unknown reason, the worms become stationary and begin to coil up, and as they do so they stimulate a fibrous tissue reaction around themselves, resulting ultimately in the formation of these subcutaneous tumours. It may be that a certain stage in the life of the worm comes when it cannot wander any more, or that while the worm is moving normally, its head or another part of its body becomes arrested, preventing further progress. It is also possible that the coils of two or more worms become twisted on each other and kinked so that the movement of the worms is arrested ; their toxic products being then concentrated at one spot, lead to tumour formation. In such cases the worm or worms instead of being spread out, start to pull up coil by coil to the point of fixation, which makes the nucleus of the tumour. In so doing a female worm, whose length may be 50 to 70 cm., would take a long time to pull itself up completely and get coiled in a ball-like mass inside the tumour. The first part of the worm which becomes stationary, the head for example, will be the starting point for the tumour. Through the toxins secreted by it and the embryos discharged, and probably also the mechanical irritation produced locally, the tissues in the immediate vicinity are stimulated to react and thus we get a series of tissue reaction changes occurring, starting by a cellular proliferation and ending in adult fibrous tissue which would be then the worm tunnel for the stationary segment of the worm. By this time one or more segments or coils of the worm would have been dragged in beside the first coil and the same series of tissue reaction changes would also occur around these ending in the formation of fibrous tunnels. This process will be repeated over and over again until the whole length of the worm is dragged in; then the process of tumour formation is complete and we find the worm or worms in the tumour intricately coiled up with the coils lying in dense fibrous canals in such a way as to make it an impossibility to dissect out a female worm without breaking it.

The above genesis of the tumours which the writer suggests is supported by the following data.

1. *The minimum sizes of the tumours.* Sharp stated that he found two tumours the size of a pin's head; on dissection, one contained numerous microfilariae but no worm was found. If we neglect this observation and consider the smallest size of a tumour to be that of a pea, which size is mentioned by various workers on this subject, then it is evident that such a tumour could not accommodate an adult female worm of from 50 to 70 cm. in length with the fibrous tunnels surrounding every coil of the worm, and the fibrous stroma of the tumour itself, which is seen between the worm tunnels and also the external fibrous capsule. Here the writer is supposing the tumour only contained one female worm, disregarding Manson-Bahr's statement which was that the tumour contains at least four males and two females. The same argument holds good for *O. gibsoni* tumours as the length of the adult female is stated to be 140 cm., i.e., more than double the length of an adult *O. volvulus* female. Small tumours are easily explained by the above theory and would then consist of a few coils of the worm surrounded by their connective tissue tunnels and the intervening stroma, and outside, the capsule. The rest of the body of the worm would be still outside the tumour mass and in the process of being dragged into the tumour. In such tumours if a circular ring of skin and subcutaneous tissue is removed all round the tumour and a little way from it and then serial sections of this skin and underlying subcutaneous tissues are made, the severed body of the still yet incompletely dragged in worm would almost certainly be seen in one of the sectors of that ring.

2. *The gradual and slow increase in the sizes of the tumours.* If the worm or worms which started the tumour were completely coiled up in a ball-like mass before the formation of the tumour began and when they became so, through their toxins and the local irritation they produce, stimulated the tissues around them to react and ultimately to become fibrosed, then the whole process of tissue reaction from commencement to completion will not take more than a month until the fibrous tissue forming the tunnel walls and stroma and capsule becomes mature: the tumour would then reach its adult stationary size in a month or so. According to the explanation given, every coil of the worm that is dragged in stimulates a

tissue reaction around it which will end in adult fibrous tissue in a month or so and this fibrous tissue contributes to the size of the tumour and the process is repeated over and over again, with gradual increase in the bulk ot the tumour until the whole worm is dragged in. Later on will be mentioned another factor that contributes to the size of the tumours even after the whole worm is dragged in and becomes encysted ; this is the irritation produced by the presence of the embryos in the tumour mass, which are discharged from time to time from the female parent. The toxins secreted by these and their parent worms plus the mechanical irritation they produce stimulate further tissue reactions in the stroma and capsule of the tumour which will end in fibrosis and thus add to the size of the tumour.

The observations of Cleland, mentioned in the first section of this paper, are important. He found an *O. gibsoni* about eleven and a half inches in length in the loose tissue of the back-part of the knee joint. This worm was not encapsulated but he found a small fibrous nodule of the size of a split pea attached to a part of the worm. The worm itself showed active movement. He also found a portion of another worm embedded in a lymphatic gland. This portion was lying in a fibrous track in the gland and surrounded by lymphocytes and also giant cells which he considered as evidence that the worm had been caught in the gland for a long time. These findings thus show that portions of the worms may be in an encysted condition with the rest of their bodies still free and showing active movement.

Different histological pictures are present in the tunnel walls around the worm sections in the tumours. The writer observed in the material examined and also in the descriptions of the various authors mentioned in the first section of this paper, that in one and the same tumour there are worm sections surrounded by tunnels, the walls of which are made up of adult mature fibrous tissue with few cells amongst the fibrous bundles, the cells being of the adult type. Other worm sections in the same tumour are surrounded by tunnels, the walls of which are made up of fibrocellular tissue which is rich in cells, some of these being round or oval, others of the adult connective tissue cell type. This means that the tunnel wall is made up of immature fibrous tissue. A third group of tunnels around other worm sections are made up wholly of cells with few or no fibrils

around them, which signifies that the tissue reaction around these worm sections is very young and of short duration.

This picture is in full agreement with the writer's explanation of the genesis of the tumours as it can be easily understood on this basis, and difficult to interpret in any other way. As a matter of fact it is this varied histological picture which was the first guide to the hypothesis about the genesis of the tumours. According to this theory the first coils of the worm which are caught in the tissues and form the nucleus of the tumour, stimulate a tissue reaction around them ; the elements of this reaction are only the proliferated cells around the worm sections and so we get a tunnel formed around the worm, the wall of which is made up of cells only. Later fibrosis occurs in these cells and so the tunnel wall becomes fibro-cellular, i.e., composed of immature fibrous tissue. Still later this fibrous tissue becomes mature with definite bundles of fibrous tissue and a few adult connective tissue cells between the fibres. So the oldest coils of the worm in the tumour would be embedded in adult mature fibrous tissue and the most recent ones would be surrounded by a cuff of reactionary cells with no fibrils amongst them and the coils that were dragged in before these last would be surrounded by immature fibrous tissues.

On the other hand if the whole body of the worm were first coiled up and then the tumour formation started we should get one stage only of fibrous tissue formation around all the coils of the worm according to the age of the tumour. If this is very recent, all the coils of the worm should be surrounded by a cellular reaction, and if the tumour is about one month old or more then the whole of the worm coils should be surrounded by adult mature fibrous tissue. Further, one would expect the whole body of the coiled worm or at least many coils of it to be lying in one worm tunnel and not every coil in its separate tunnel as is seen in the sections. In other words, there would be little or no fibrous tissue between the coils of the worm.

(5) In one of the sections of the *O. volvulus* tumour which was examined there was found a portion of a female worm cut longitudinally and a part of it lying outside the capsule of the tumour, the rest piercing this fibrous capsule obliquely and lying in a tunnel, the wall of which was made up of the dense fibrous bundles of the capsule

externally and internally by a cellular layer lying next to the cuticle of the worm. The tunnel with the worm inside it pierced a large part of the thickness of the capsule. The worm was an adult female containing embryos in its uteri.

This again is an important observation which supports the above theory. It will be remembered in the writer's description of the tumour, that it was a small one measuring only about 1 cm. in diameter: it was therefore, a growing one and had not reached its mature size. Further, in its histology there were described worm sections which were surrounded by tunnels, the walls of which were made up by a thick layer of reactionary cells with no fibrils amongst them, i.e., these tunnel walls were very recent in their formation or, in other words, the worm coils inside them must have been dragged into the tumour quite recently. Thus, the whole length of the worm was not inside the tumour; a part of it was still outside. There must be a canal in the wall of the capsule of the tumour for the rest of the worm body to pass through to the inside. The above observations are thus in harmony with that theory.

THE HISTOGENESIS OF THE ONCHOCERCAL TUMOURS

It seems that the tissue reactions excited by the toxins liberated from the worms and embryos follow a definite course which is not peculiar to the toxins of Onchocerca, but is shared by those of other helminths as will be shown, in dealing with the tissue changes which they excite, in a later section. Thus when a worm section or coil becomes stationary in the tumour, it excites, through its toxins, a cellular reaction which consists of large giant cells appearing immediately outside the worm cuticle and then by many layers of endothelioid cells with numerous plasma cells. No fibrous elements are to be seen at this stage. Later on eosinophile cells begin to appear heavily among the above-mentioned cells and also in the tissues around. With the appearance of the eosinophile cells a process of fibrosis starts at this young cellular reaction tissue. This fibrous transformation commences at the periphery of the cellular tissue and continues concentrically inwards till all the cellular tissue is densely fibrosed, with the result that a worm tunnel is formed around the section of the worm, composed of whorls of adult fibrous tissue

bundles with few adult connective tissue cells among the fibres. This series of reaction changes repeats itself over and over again around every newly arrived worm coil in the tumour until the whole length of the worm or worms is dragged in and ultimately surrounded by adult fibrous tissue canals. This histogenesis of the tunnel formation explains the varied microscopical pictures exhibited around the worm sections in the tumours.

The different microscopical pictures which are noted in the stroma of the tumour in between the worm tunnels are explained on the same general basis mentioned above. We know that the worms inside the tumour, like the free adult wandering worms, discharge, from time to time, living embryos which stay for a certain time at least in the stroma of the nodule before finding their way out of it, if ever they do so. As the embryos wriggle about in the nodule they discharge their toxins. The adult worms, as long as they live in the tumour, also discharge their toxins which will diffuse through the worm tunnels around them into the intervening stroma. These toxins of both adults and embryos when they reach a certain concentration in any part of the tumour, excite in that area a series of tissue reaction changes exactly similar to those that occur in the formation of the worm tunnels, thus starting by islands of endothelioid cells with some giant cells and plasma cells among them. Sooner or later eosinophile cells are mobilised to that area in large numbers if the concentration of the toxic products is great, and then a process of fibrosis starts and ends in adult fibrous tissue with fibroblasts among these and the above cellular elements disappear. The giant cells which are sometimes seen in large numbers in the stroma serve the function of removing the remains of the effete and dead bodies of the microfilariae that succumb.

By analogy of what happens around tubercular deposits, the writer believes that the body of the host, in its attempt to get rid of the parasites and their toxins or to minimise the diffusion of the latter into the surrounding tissues, is stimulated to react locally around these growing tumours with the result that a fibrous capsule is formed which shuts the parasites inside it, and, as it grows denser and denser, it becomes more and more non-vascular with the ultimate result that a certain stage comes when very little nutrition goes to the parasites inside and thus they die.

The thickening of the vessels which is very marked especially in the capsules of *O. gibsoni* tumours, and which was noticed by Sharp in one case of *O. volvulus*, is due to the diffusion of the toxins locally into their walls from the tumours and also to the toxins secreted by the embryos which find their way into the walls of these vessels. Cleland in one of his illustrations showed a blood vessel with two embryos lying in the actual wall of the vessel. The writer finds it difficult to agree with Sharp that this thickening is due to larvae circulating in the blood, as the evidence points to this circulation of larvae being an infrequent occurrence. It appears easier to explain this as a local reaction of the vessel wall due to toxins. Gilruth's observations that greater thickening occurred in the older tumours might be capable of interpretation on either the larva circulation, or the local toxin hypothesis. But it is evident that if the thickening is due to the effects of circulating larvae, it should be present all over the body and not only in the tumour or the larval skin sites. This is a point which it would be of interest to determine.

THE SECONDARY CHANGES WHICH MAY TAKE PLACE IN THE ADULT TUMOURS

The size of the full-grown tumours varies very much as is described by various authors. The variation in the sizes of these tumours may be due to the fact that the bigger ones contain a greater number of worms with a correspondingly greater amount of fibrous tissue around the worms and in the stroma than the smaller tumours. The fact that some of these older tumours contain more fluid in them which is of a darker colour or that some of them become cystic, may be explained on the basis that as the tumour gets old, the fibrous tissue it contains, including the capsule, becomes mature, dense and relatively avascular and as the blood and lymph supply from which the worms derive their nutriment likewise become diminished, the worms then die and their bodies undergo a sort of colliquative necrosis resulting in the formation of cystic spaces and an increase in the fluid contained in these tumours, the dark colour being due to the liquefaction of the bodies of the worms, while the contained ova and embryos are set free in the fluid ; these ultimately die and liquefy too. Again, the bodies of the dead worms may become calcified as occurs in *W. bancrofti* when this dies and is not completely absorbed.

Another possible termination is that the worms contained in a tumour die early, e.g., as a result of trauma and before the tumour becomes fully fibrosed and avascular; it would be quite likely that the bodies of these worms would act as inert foreign bodies and thus stimulate phagocytosis around them with the formation of foreign body giant cells ; thus the whole of the bodies of the dead worms may become completely absorbed and replaced by granulation tissue. This ultimately becomes transformed into adult fibrous tissue with no trace of the worms in it and the whole tumour would then look macroscopically and microscopically like a fibroma. This may explain the occurrence of juxta-articular nodules in the endemic areas of volvulus, especially if we consider the fact that the tumours around the joints are more exposed to pressure and injury than at any other region of the body. Here the writer agrees with Blacklock and Sharp in the origin of some at least of the juxta-articular nodules.

The observations of Blacklock mentioned in the first section of this paper are important and support this possible termination. Thus in his first case, although the skin was positive for larvae, the seven nodules around the elbow and knee gave negative results on puncture, which means that the worms in these tumours were most probably all dead. Similarly, in his second case, which exhibited four tumours around the right knee-joint, the worms were dead in three of these and alive in the fourth. In these old tumours the fibrous tissue of the capsule and stroma may undergo shrinkage like scar tissue anywhere in the body, resulting in a diminution of the sizes of the tumours.

Many authors have reported the frequent occurrence of adhesions between the tumours and the surrounding tissues such as the subcutaneous connective tissue, muscles or underlying bones. These adhesions are most probably due to the toxins in a concentrated form or the microfilariae inside the tumour, gaining access to the surrounding tissues where they excite inflammatory phenomena which end in adult fibrous tissue binding the tumour by adhesions to the surrounding structures. That the larvae are in the habit of penetrating the intact capsule of the nodule seems possible as shown by a series of experiments conducted by Nicoll on nodules excised from the carcasses of slaughtered cattle. The nodules were examined very carefully before the experiment to ensure that no cuts were

present in the capsule. Nicoll concluded his work by stating that the larvae can and do make their escape through the capsule of the worm nodule. They usually did so in small numbers but in some cases comparatively large numbers migrate. It will also be remembered from the histology of the *O. gibsoni* tumour that the capsule was heavily infected with embryos and it is quite possible for these and their toxins to go to the tissues outside the capsule.

With regard to the histology of these tumours the writer believes that so long as the worms inside are alive and especially the fecundated females which discharge embryos from time to time, there will be zones in the stroma of the tumours and perhaps in the capsules also, which will show a greater or lesser amount of cellular reactionary tissue and eosinophile cells which are the result of the reaction against the toxins secreted by the worms and embryos. At the same time, as a result of this continuous addition of fibrosed tissue, the tumours, although all the length of the worms is dragged in, will exhibit a slow and secondary increase in their size. On the other hand, when all the worms and their progeny inside the tumour die, the cellular tissue in the stroma or the capsule will become fibrosed; then a section of the tumour will show the remains of the worms intact calcified, liquefied, or perhaps absorbed, and bundles of adult fibrous tissue around these and in the stroma and capsule, with a few adult connective tissue cells. Meanwhile the eosinophile cells will have disappeared.

ANALOGY OF THE TYPE OF TISSUE REACTION EXHIBITED IN ONCHOCERCAL TUMOURS TO THOSE EXCITED BY SCHISTOSOMIASIS AND FILARIASIS

Having some experience in the morbid histological changes exhibited in bilharzial deposits, the writer was deeply impressed with their great resemblance to the histological changes present in onchocercal tumours. Thus the various stages described of the microscopical appearances around the worms in the tunnel walls and also in the stroma of the tumours, especially the *O. volvulus* one, are reproduced in bilharzial deposits around bilharzia ova practically without deviation of type. They start from the cellular stage, which consists of endothelioid cells, giant cells and plasma cells;

then the appearance of the eosinophile cells is followed by a process of fibrosis commencing from the periphery and continuing concentrically until the whole cellular reaction tissue is completely fibrosed. The following short account of the writer's observations in schistosome lesions will make this quite clear to the reader.

In describing the pathological changes excited by the deposition of schistosome ova in the tissues, they were divided into two types of tissue reaction : (1) the diffuse type, and (2) the nodular type.

In the diffuse type, the ova are more or less diffusely distributed, producing a diffuse tissue reaction. The elements of this tissue reaction differ with the age of the lesion. In the younger ones it is distinctly cellular consisting of endothelioid and plasma cells with no eosinophiles. In slightly older lesions eosinophile cells are added to the above mentioned elements and after the appearance of these a fibroid transformation starts in the cellular tissues which continues till complete solid fibrosis is established. This is seen in very old lesions where the ova have become calcified or destroyed by giant cells and disappeared. In such chronic lesions no eosinophile cells are seen.

In the nodular type the ova are deposited singly or in small groups of two or three at most. The youngest ova are seen surrounded by many layers of typical endothelial cells with the formation among them of large giant cells immediately surrounding the ova or a little way from them. These discrete tubercle-like nodules are seen embedded in a fibrous matrix. Up to this stage no eosinophile cells are to be seen, but later on heavy eosinophile cell infiltration appears in the fibrous matrix surrounding the nodules. Contemporary with the appearance of the eosinophile cells, a fibroid transformation commences in the peripheral layers of the endothelial cells which continues concentrically inwards until the nodules become completely transformed into whorly layers of fibroid tissue. Occasionally the eosinophile deposits are very heavy indeed, especially around the healthier looking non-calcified ova.

Thus from the above resumé of the histo-pathology of schistosome lesions, the reader can see at once the great similarity between these and the onchocercal lesions. Sorour's findings in the lesions of Schistosome disease coincide with the above description.

In the affection of the blood vessels schistosomiasis does not

fail to show the analogy. Sorour observed that if a bilharzia worm dies in a vein, proliferation of its endothelial lining takes place round the lumen in a marked way. This predisposed to thrombosis, while the potent new tissue-forming bilharzial toxins encouraged the organisation process which is much denser in these bilharzial thrombi than any other thrombus organisation. The bilharzia ovum, he stated, is also capable of producing marked lesions in the walls of blood vessels, lesions that are indeed exactly similar to the well-known vascular lesions found in syphilitic and arteriosclerotic cases with no bilharzial element. Thus if a bilharzial ovum remains in a small blood vessel, it excites proliferation of the endothelial lining round the lumen which gradually narrows until it just holds the irritating ovum. This is a pure endothelial lesion. If the ovum in the lumen of the vessel is partly engaged with its spine in the intima so as to touch the subendothelial layer, the latter tissue proliferates but only in the sector where the bilharzia ovum is engaged. The lesion produced is a purely subendothelial one and resembles exactly the local arteriosclerotic lesions found elsewhere. When the ova obtain complete access to the subendothelial layer they produce great proliferation of the tissue with giant cells and eosinophiles and the lesion formed would be exactly similar to that of ordinary arteriosclerosis were it not for the giant and eosinophile cells. If the ovum is caught in the muscular wall of the vessel it produces a nodule in that site resembling a healed aneurysm.

From the above account it is quite evident that bilharzia produces thickening of the walls of the vessel which are very similar to ordinary arteriosclerotic lesions. The writer has seen the actual slides of Sorour and the illustrative microphotographs taken from them.

The histological pictures of the reaction tissue presented in onchocercal tumours and schistosome deposits are reproduced in the reaction tissue excited by *W. bancrofti*. Chronic nodular masses varying in size from a hazel nut to a pigeon's egg are occasionally met with in the spermatic cords of the Egyptian peasants. These are found to be due to either schistosomiasis or to filarial infections. Sorour found that the filarial nodules showed chronic fibrosis with heavy eosinophile cell infiltration, enclosing thick-walled blood vessels and intact filaria worms with ova or embryos or dead and degenerated worms inside lymphatic vessels ; these in some cases

showed layers of endothelial cell proliferation containing many multinucleated cells and eosinophile cells surrounding the worm in the vessel. In other cases he found the degenerated or calcified worm lying inside a fibrosed vessel.

Sorour concludes that the filarial toxins also produce endothelial cell proliferation with multinucleated cells and a local heavy accumulation of eosinophiles and here the end result is a chronic fibrotic lesion. Thus again the same picture is reproduced by the filarial toxins.

The writer has limited himself to showing that the histopathological changes excited by *Onchocerca*, *W. bancrofti* and the Schistosomes are exactly similar to each other. It is probable that the same picture will be found reproduced by all the helminthic deposits in the tissues irrespective of the class to which the worm belongs. This reaction should therefore occur in the tissues in trichinosis, around hydatid cysts and the cysts of *Cysticercus cellulosae* and *C. bovis*. This would indicate that the helminthic toxins form a group of closely allied toxins as regards the tissue reactions they produce in the host.

In studying the descriptions of the tissue reactions in trichina and cysticercus it is not always possible to determine what cells exactly were meant by the authors, although they almost invariably made particular mention of the eosinophile cells.

THE HELMINTHIC TOXINS

Some authors mention these toxins with caution as if doubtful of their existence ; some even deny their existence at all and ascribe the symptoms produced in the host of these helminthic infections to other secondary factors. These are the mechanical irritation produced by the parasites in the intestine or tissues, or the loss of blood due to the bites of the worms in the intestine as in ancylostoma, which leads to anaemia ; or again the mechanical injury and loss of blood and anaemia produced by the piercing of the schistosome ova through the mucosa of the bladder and intestine. It is suggested even that the symptoms are due to the absorption of part of the host's nourishment by e.g. Ascaris. It is true that these secondary phenomena do produce a certain amount of ill-effects and contribute

to the symptoms produced, but these are, however, mainly due to the toxins secreted or excreted by the parasites.

The helminthic parasites do actually secrete or excrete toxins which act deleteriously on their hosts producing general or local symptoms or both, according to whether the parasites and their ova or embryos live in hollow organs such as the intestine and the interior of blood and lymph vessels or actually invade the tissues. The variation in the severity of the symptoms produced in the host by the different helminths or even by the same helminth in different patients is due to the fact that some helminths secrete more toxins than others and that these toxins have a better chance to reach the circulation. Thus in comparing ancylostoma and ascaris infections, the ancylostoma toxins are discharged directly into the tissues while the worm is feeding from the mucosa of the intestine, whereas in ascaris the majority of the toxin passes out with the faeces and only a part of it is absorbed as the worms do not injure the intestinal mucosa. Again the toxin of one parasite may be more potent and strong in its action than the toxin of another parasite. In the cases in which the symptoms produced by one parasite are severer in one host than another, this is often due to the difference in the severity of infections, one host being infected by a larger number of parasites. This phenomenon is well illustrated by the ancylostoma infestations. These helminthic toxins do produce general and local symptoms and in this way they behave more or less like the bacterial toxins. Thus in the majority of the helminthic infections the patients develop a fever especially during the early phases. This is seen in trichinosis, schistosomiasis, onchocerciasis, etc. As a reaction on the part of the host, an eosinophile cell leucocytosis soon develops and these cells migrate in large numbers to the local foci in the tissues, if the invading helminths happen to lay any deposits in these as in trichinosis and schistosomiasis. The eosinophilic leucocytosis in the blood and tissues lasts so long as the helminths and their deposits are living in the host and the eosinophile haemopoietic system is not exhausted and is ready to react. This reaction of fever and eosinophilic leucocytosis excited by the helminthic invasion is exactly similar to the reaction of the body against the toxins of any bacterial infection with the exception that here the leucocytosis is eosinophilic instead

of being neutrophilic. Later on, according to the severity and duration of the infection secondary degenerative changes may set in, in the parenchymatous cells of highly specialised organs, as the fatty degeneration that occurs in the kidneys and the liver in heavy ancylostoma infections, and in fatal cases of trichinosis. Again oedema of one or more regions of the body may set in as in ancylostoma where oedema may occur in the face and later in the feet and ankles and other parts of the body. Also in trichinosis where the patient may develop oedema of the eyelids and face and later oedema of the limbs. In *O. caecutiens* too, the patient sometimes develops oedema of the face. Later on a certain degree of anaemia develops with asthenia and frequently some mental deterioration.

Again, when there is a helminthic deposit in the tissues as in schistosomiasis, onchocerciasis, filariasis and trichinosis, the tissue reactions excited locally as a result of the toxins liberated from the worms or embryos follow, as shown in the previous section of this paper, a certain definite chain of processes; these start by endothelioid cell proliferation and giant cell formation and later, by eosinophile invasion and then fibrosis in these cellular elements until the whole cellular reaction tissue is transformed into a nodule of adult mature fibrous tissue, with the irritating worm, ova or embryos in the centre if they are not already absorbed or calcified. Thus the helminthic toxin in these reactions shows itself to be a potent endothelial cell excitor, behaving in this phenomenon exactly like that of the tubercle bacillus. That these specific tissue reactions are excited as a result of the helminthic toxins locally and are not simply due to mechanical irritation of the tissues by these deposits, is proved by a simple experiment conducted by Sorour; he showed that living schistosome ova produced bilharziomata in the lungs when injected into the ear vein of the rabbit, while ova from the same source, intact but killed by heating, failed to produce lesions when similarly injected. This simple experiment also proves that the toxins are not only secreted by the adult worms but even by the embryos inside the egg-shell. Onsy (1930) made subcutaneous injections of emulsions of dead bilharzia worms and this was followed by pronounced eosinophilia both locally at the seat of the injection and generally in the circulation, which again proves the reaction of the body against the toxins present in the

body of the worm. Shimamora and Fujii recovered what they termed an albumose peptone from the fractionation of the aqueous extracts of *Ascaris lumbricoides* and *Parascaris equorum* which gave various symptoms of poisoning when injected into horses, guinea-pigs, dogs and rabbits. They called this poisonous principle ' crude askaron.'

From this brief account, we can conclude that the helminths do secrete toxins and that the general and local symptoms exhibited in the host are mainly due to the deleterious effects of these toxins.

THE INFECTIVE GRANULOMATA AND THE HELMINTHIC DEPOSITS IN THE TISSUES

There is a great deal of similarity between the tissue changes produced by the infective granulomata and the helminthic tissue changes which result when the helminthic parasites or their ova or embryos are permanently deposited in the tissues, as in schistosomiasis, filariasis, and onchocerciasis.

The granulomata form a group of pathological conditions in which the lesions are the result of subacute or chronic inflammatory changes associated with various proliferative and reparative processes. They may produce considerable masses which in many respects resemble tumours. Further they are almost always associated with various toxic conditions such as fever, wasting and fatty degeneration. These granulomata or granulation tissue tumours are caused by known infective agents such as the tubercle bacillus, and in syphilis, leprosy, glanders, and the various affections with streptothrices as actinomycosis. Hodgkin's disease or lymphadenoma, although its cause is still unknown, is also allied to these granulomata.

The granulomata produce a series of tissue reactions which are more or less specific and diagnostic of the causal agents, and, taken as a group, form a variety of diseased conditions which excite tissue changes allied both macroscopically and microscopically. Thus in their microscopical appearances a number of them commence their tissue changes by endothelial cell proliferation with giant cell formation. This is followed by leucocytic infiltration of the tissues around. The leucocytes vary in the different diseases, e.g. being lymphocytes in tuberculosis and syphilis, and eosinophiles in

lymphadenoma. In some cases caseation occurs, as in syphilis and tuberculosis, but in many cases the process ends by fibrosis of the cellular elements before or after the caseation, thus producing a fibrous or fibro-caseous nodule as the case may be. The nodules may be large enough to simulate actual tumours.

Reference is here made to the histogenesis of the miliary tubercle to show its similarity to the helminthic lesions. The tubercle bacillus, after it settles in the body, multiplies and injures the tissues, but it stimulates the fixed connective tissue cells and the endothelial cells into proliferation. In this way a non-vascular nodule appears composed of large endothelioid cells with many giant cells. In from seven to ten days round cells begin to appear, sometimes in great numbers, coming from the surroundings and resting at the periphery of the tubercle, and often in great abundance between the epithelioid cells. They are mostly lymphocytes with some plasma cells. Isolated polymorphonuclear leucocytes may appear too. During this stage the blood shows a lymphocytosis. The focus sooner or later disintegrates and becomes caseous especially in the centre, the caseation being due to the necrosing influence of the tubercle toxins which injure newly-formed cells and also prevent the formation of connective tissue and blood vessels. Under favourable circumstances, however, no caseation of the cells occurs and the whole nodule becomes fibrosed completely, or even after the caseation has commenced and continued for some time, fibrosis of the endothelial cell layer surrounding the caseous mass occurs thus shutting this inside a fibrous capsule.

The toxins of the tubercle bacillus excite what we may call a form of productive inflammation starting with endothelioid and giant cell proliferation, with later lymphocytic cell invasion and ending in fibrosis. It is thus very similar to the productive inflammation excited by the helminths in the tissues with this difference, that the eosinophile cell of helminthic infection is lacking while its place is taken by the lymphocyte in tuberculosis. The structure of the syphilitic gumma is very similar to that of the tubercle with some slight but diagnostic differences. The caseation which occurs in tuberculosis is due to the great toxity of the tubercular products. In the foregoing description of the *Onchocerca volvulus* tumour it was shown that there are areas, where the endothelioid cells around some worm tunnels, especially where there are numerous tunnels lying

nearly side by side, showed degenerative changes and this was attributed to the greater concentration of the toxins secreted from the worms locally.

The infective granulomata produce large masses resembling tumours which are called granulation tissue tumours. Examples are the large tubercular masses which occur in the lymph glands or any other part of the body and also the syphilitic gummata and leprous and glanders nodules.

Those helminthic parasites and their ova or embryos which invade or become deposited in the tissues do sometimes, as a result of the irritation they produce by their toxins, give rise to large masses resulting from the proliferative tissue reactions they excited ; these masses resemble tumours. We have already seen this in the onchocercal tumours and in the tumour nodules mentioned above under parasitic funiculitis in the Egyptian peasants which are found to be due to *W. bancrofti* or schistosomes. In other lesions of schistosomiasis we sometimes find what Madden (1919) called bilharzial tumours occurring in the large intestine ; in the caecum, transverse colon, and especially the sigmoid flexure. These tumours usually involve a length of about four to eight inches of the intestine ; they are very large and palpable through the abdominal wall and all the coats of the colon are thickly infiltrated with schistosome ova and bilharzial granulation tissue. Thus from within outwards, we find the mucous membrane very thickened and from it hang large cauliflower-like pedunculated papillomata which nearly block the lumen of the gut ; the muscular coat is also greatly thickened by the same bilharzial granulation tissue around the deposited ova ; the subperitoneal and peritoneal coats share in this reaction. In many cases, the meso-colon and appendices epiploicae also become infiltrated and thickened and even the glands in relation to that area become enlarged and infiltrated. Thus the picture resembles a sarcoma of the intestine. Independent, large, cauliflower-like papillomata may occur also—the mucosa and submucosa alone being involved—in the colon or in the rectum and may be even prolapsed from the anus. Schistosome papillomata of the urinary bladder and other parts are very frequent. The reader is here referred to Madden's ' Surgery of Egypt,' where he will find photographs and other details of schistosome granulation tissue tumours.

Thus, in the helminthic invasions of the tissues, as in the infective

granulomatous diseases, we get, as a result of the irritation of these tissues, subacute and chronic proliferative and productive inflammatory phenomena which may go on to the formation of large tumour-like nodules. The inflammatory phenomena which take place in these helminthic deposits are quite different from the phenomena of acute inflammation and repair in the general inflammations which are excited, e.g., by the pyogenic bacteria, where the process leads to destruction of tissue and the repair is limited to the filling-in by fibrous tissue of the gaps left. The writer believes that the endothelioid cell plays a very important rôle in the defensive mechanism of the body against its invasion by the helminthic deposits or in the granulomata as tuberculosis or syphilis. Thus, besides its phagocytic and perhaps other properties, by its proliferation, e.g. around a tubercle bacillus or a schistosome ovum, it makes an avascular nodule around these which serves the double purpose of cutting off the food supply and drenching them in their own toxins, both processes being inimical to the life of these noxious invaders.

THE PATHOLOGICAL MANIFESTATIONS EXCITED BY *ONCHOCERCA VOLVULUS* OTHER THAN THE TUMOURS

The clinical and pathological pictures attributed to *Onchocerca volvulus* vary greatly in the different endemic centres. Thus in the Congo, where *O. volvulus* infection is common, Ouzilleau (1913) described, besides the tumours or cysts in patients, the occurrence of cutaneous lesions such as elephantiasis of the scrotum and other regions, pseudo-ichthyosis, ichthyosis simplex and lichenification. He described also the frequent occurrence of enlargement of the glands and hydrocele. In this area, he stated, *Filaria bancrofti* is absent and *Onchocerca volvulus* present.

Sharp (1926) in Nigeria described, besides tumours, the frequent occurrence of infection of the groin and other glands, hydrocele, marked localised thickening of the skin, lichenification and every variety of craw-craw.

Blacklock (1926) in Sierra Leone in the Konno District, where *O. volvulus* is very prevalent, stated that neither in the case of the eyes, skin, or the glands did the patients show pathological effects solely and definitely due to *O. volvulus* infection ; but he thinks it

may be that in localities where hyperinfection with the worm occurs, a different clinical picture may be produced.

Further, Blacklock and Sharp are agreed on one point, the continuous presence of the larvae in the skin of the patients even in those cases which exhibited no nodules, so that the skin examination always gave a higher positive result than the examination for tumours. This can only mean that the worms lead a free life in the tissues and as they wander about discharge embryos which invade the skin.

A point not cleared up by the various writers is which skin structures form the habitat for the embryos; i.e. whether it is the skin, blood capillaries, or the capillary lymphatics or whether they live among the tissue cellular elements. Thus, Ouzilleau, Laigret, and Lefrou do not specify their exact situation nor did Blacklock do so. He showed, however, that they were not present in the cutaneous blood. Sharp stated that the embryos were seen in the various layers of the cutis vera up to the apices of the papillae. Even this statement does not help us much to detect their exact location. Reference is here made to the minute anatomy of the vascular and lymphatic distribution in the tissues including, of course, the skin, as this may help in our discussion. It is generally believed that in no part of the body does the blood come in actual contact with the living cells of any tissue. In all the tissues the blood flows in capillaries with definite walls consisting of a single layer of cells, and is thus separated from the tissue elements by these walls and a varying thickness of tissue. The middleman between the blood and tissues is the tissue fluid or tissue lymph which fills the spaces between all the tissue elements, so that any tissue can be regarded as a sponge soaked in lymph. Throughout these spaces we find a close network of vessels lined and separated from the tissue spaces by a layer of extremely thin endothelial cells and this plexus communicates with definite channels, the lymphatics, by which any excess of fluid in the part is drained off. Most of the lymphatics drain into the thoracic duct which pours its contents into the venous system at the junction of the left jugular and the subclavian veins. Thus the lymph is a part of the blood plasma which exudes through the capillary walls, bathes all the tissue elements and then passes between the endothelial cells into the peripheral lymphatic network whence it is carried by lymphatic trunks into the thoracic duct by

which it is returned again to the blood. Having sketched the circulation of the blood and lymph and their relation to the tissue cellular elements, the next point is to try and discover the actual habitat of the embryos among these anatomical units. The writer believes that the embryos do not live in the peripheral blood capillaries nor in the peripheral lymphatic capillary network, but that they live in the above-mentioned tissue spaces which are bathed with lymph and which surround the actual tissue elements or cells. The facts that support this theory are the following :—

The majority of the workers on the subject reported the negative finding of the embryos in the blood and those who reported their presence said this was very rare and exceptional.* If the embryos lived in the blood or lymph capillaries of the skin they would certainly be washed down in these streams and appear in the general circulation at some time. Blacklock's observations showed that the infection of the lymphatic glands which drain the cutaneous lymphatic capillaries, did not present itself as a noticeable feature in his *volvulus* patients. Naturally if the embryos lived in the lymphatic capillaries some of them at least must be washed down or wriggle about until they arrive at the lymphatic glands draining these infected areas of the skin. Further, Blacklock noticed that the infection is not uniformly distributed all over the skin surface of the body but that it is localised to certain areas and that some of these show hyperinfection more than others. Now if the larvae were in the lymphatic vessels, after traversing the length of the lymphatic trunks, and appearing in the blood, they would be seen in the skin capillaries anywhere and not only in certain areas. The embryos most probably live in these tissue lymph spaces which form a sort of sponge with no regular or formed channels ; they cannot therefore travel very far from the spot where they were discharged from the parent worm. In mild infections there is plenty of room for them in these tissue spaces, so that they move

* The highest figure for the presence of embryos in the blood was recorded by Sharp to be 8 per cent. in a series of over 2,000 cases. The writer's comment upon this high figure is that in order to reach the blood in the skin, the needle has to pass through the embryo infected area and so these are apt to be dislodged and are drawn out with the blood. The safest procedure would be to choose an area of the skin which proves to be uninfected with embryos and then to draw the blood by vein puncture with a syringe containing about 1 c.c. of sterile saline solution, which should be injected first into the vein, as an accessory precaution to wash away any embryos that may have come on the tip of the needle while piercing the skin, and then to draw the blood into the syringe and examine it.

about quite freely, but in cases with hyperinfection where the patient is infested with a large number of adult females, which in turn would mean myriads of embryos to be discharged, the tissue spaces become so choked with the embryos and their motility becomes so difficult that they tear through the very delicate layer of endothelial cells which forms the walls of the peripheral lymphatic capillaries and thus gain access to the interior of these channels. They are then sucked up to the lymphatic glands which try to stop them. Some may overcome this barrier and find their way to the general circulation. It is quite probable also that some of the embryos may find their way direct to the general circulation by piercing the wall of the capillary blood-vessels in the hyperinfected areas of the skin.

Another point that supports this theory is that in the tumour sections which the writer examined and also in the descriptions and microphotographs of some observers, the embryos are never seen inside capillary blood vessels or lymphatic channels with an endothelial lining. They are seen in the empty clefts between the cellular elements or in other words in the tissue lymph spaces.

It may be noted here that the embryos can find the richest supply of nutriment in the tissue lymph as this is coming direct from the circulation and the food elements are not yet used up by the tissue cells.

To return to the cutaneous lesions mentioned by the various authors at the beginning of this section, the writer agrees with Blacklock that the skin reaction produced by onchocercal infestations varies with the severity of the infection. Thus in mild infections of the skin where the embryos are not very numerous they live there producing no symptoms and the skin may be quite normal in appearance. The toxins which are secreted from these embryos are diluted by the tissue lymph and absorbed and pass to the lymphatics and general circulation immediately they are formed ; the concentration of these dilute toxins is insufficient to stimulate the tissues to react locally. The picture is totally different in hyperinfections. Here the embryos are in enormous numbers, the amount of toxin secreted by them is great and so the tissue lymph becomes saturated with them to a degree sufficient to stimulate the local tissues to react in exactly the same way as we have seen

in the formation of the tumours. We therefore get a process of fibrosis commencing by a certain amount of endothelial proliferation and emigration of eosinophiles and ending by mature fibrous tissue. This same process is repeated over and over again with every discharge or invasion of new embryos and each time we get more fibrosis. Meanwhile the skin structures proper are destroyed and the fibrous tissue is formed each time at their expense.

Thus after these attacks have been repeated many times a greater or less amount of fibrous tissue is formed locally and this accounts for the localised thickened areas of the skin. Also the lymphatic capillary network which lies in the tissue lymph spaces and even some of the lymphatic vessels in the area may become destroyed and obliterated. This explains the cases of elephantiasis, lymph scrotum and hydrocele.

Again the pigment cells of the skin may be destroyed, causing the patient to develop leucodermia, and the sweat and sebaceous glands may be destroyed in a similar way. The frequent infection and enlargement of the glands in certain districts is explained by the fact that there is a hyperinfection prevailing and that the embryos, which are overcrowding the tissue lymph spaces of the skin, tear their way through the lymphatic capillary network lying in these lymph spaces and so reach and infect the glands draining the area.

Moreover, the presence of the embryos in large numbers in the skin and also their concentrated toxins may irritate the nerve endings and thus lead to scratching, and the patient develops some of the lesions described under craw-craw, especially after secondary septic infection sets in. From a pathological point of view, the writer believes that the only skin lesions which could primarily be attributed to onchocercal hyperinfections locally are elephantiasis, localised thickened areas of the skin, leucodermia and pruritus. Any other skin lesions that may show themselves in the infected areas are only *secondarily* due to onchocerca infection through the pruritus and scratching and superadded pyogenic and other microbic infections.

If the reader accepts the view put forward in this section as regards the natural habitat of the microfilariae in the tissue lymph spaces of the skin, it can be readily understood that the patho-

genesis of the onchocercal elephantiasis differs a great deal in its essential phenomena from that of the elephantiasis commonly met with in *W. bancrofti* infections. The generally accepted explanation of the mode of origin of elephantiasis in *W. bancrofti* is that it is caused by bacterial infection, usually streptococci ; *W. bancrofti* being one of the many causes giving rise to it through lymphatic obstruction, thereby lowering the resistance of the tissue to a predisposition for bacterial invasion, the bacteria in turn causing the lymphangitis, which is repeated several times and ends ultimately in elephantiasis. Thus in this form of elephantiasis the presence of bacteria seems to be essential and causes repeated attacks of lymphangitis or inflammation of the lymphatic vessels and ends in a complete blockage of these vessels and elephantiasis ; consequently the lymph drainage apparatus of the area is put out of order. Fülleborn and Simon (1913) stated that if onchocerca developed in the lymphatic vessels, which seems to be quite possible, there would surely result a great deal of lymphatic obstruction, because there would be in addition to the larger nodules accessible to palpation, more numerous smaller ones which cannot be diagnosed clinically at all. Fülleborn (1924), who believes the above-mentioned mode of causation of *W. bancrofti* elephantiasis, thinks it quite possible that *O. volvulus* contributes to the production of the elephantiasis that is associated with it, in a way similar to the rôle of *W. bancrofti*, i.e. by obstruction of the lymphatic vessels by the numerous small volvulus nodules which grow and block them.

In onchocercal elephantiasis, however, repeated attacks of lymphangitis are not often reported. There would be no necessity for the bacteria or for lymphangitis. The saturated toxin of the embryos in the tissue lymph spaces, being a potent endothelial excitor, would stimulate the very delicate endothelial cells forming the walls of the capillary lymphatic network in the tissue lymph spaces to proliferation ; this would end in fibrosis and partial obstruction of the lymphatic network. After many repeated acute attacks the whole of this network would be obliterated and so the lymphatic draining apparatus of the part will be put out of order and by this time elephantiasis would have declared its presence.

So, while the lymph block in bancrofti elephantiasis occurs in the lymphatic vessels or trunks or even in the lymph glands after many

attacks of lymphangitis caused by bacteria, the lymph block in onchocercal elephantiasis most probably occurs in the capillary lymphatic network in the tissue lymph spaces after repeated attacks of inflammation caused by the concentrated toxins secreted by the embryos locally in these spaces, without any necessity for any bacterial invasion or inflammation of the lymphatic vessels draining the part. Ouzilleau, Laigret, and Lefrou reported that in the elephantiasis lesions due to *O. volvulus* microfilariae swarmed in the dermis and spread into the hypodermis.

ONCHOCERCA CAECUTIENS

This was discovered by Robles in Guatemala in 1915, who found nodules and a filaria like *O. volvulus* with embryos, and attributed to it frequent eye symptoms associated with the nodules and also *erysipela de la costa* (coast erysipelas). Robles sent some of his material to Brumpt who confirmed his findings, but created a new species *Onchocerca caecutiens* Brumpt, 1919 (the blinding filaria). Brumpt regarded it as a new species mainly on account of (*a*) association with a curious punctate keratitis and other eye lesions which disappear with dramatic suddenness after the removal of the nodules from the head ; (*b*) minor differences in the morphology of the male parasite and the predilection of the tumours for the head (they occur, according to him, on the head in the American species, in 99 per cent. of the cases, whereas in the African species they occur on the head in 1 per cent. of the cases) and (*c*) the endemic zone of the disease being at an altitude of 600 to 1,200 metres. He found that the female reached the size of about half a metre.

According to Castellani, in a well-marked case, several nodules each the size of from a pea to a large nut are seen on the scalp though less frequently they may be found also on the body especially on the sides of the chest close to the ribs. In erysipelas of the coast which is often associated with the presence of the tumours, the patient suddenly develops a high temperature which remains for two or three days when it rapidly falls to normal. At the same time, while the temperature is rising and while it lasts, the skin of the face, scalp and ears becomes oedematous and rather hard. There is no redness

as in ordinary erysipelas. The oedema gradually disappears after the temperature has become normal, but the skin of the face and especially on the forehead remains somewhat rough and occasionally slightly thickened.

The eye lesions besides the oedema of the eyelids are keratitis punctata and Calderón (1920) suggested that it produces lesions in the iris and cornea as well. Photophobia, xerosis and impaired vision result or the pupil may become obliterated and complete blindness ensue.

Fülleborn (1923) doubts whether this new species of filaria created by Brumpt is valid, as he sees no morphological differences between *O. caecutiens* and *O. volvulus*. He also does not believe that erysipelas of the coast and the eye lesions are in any way connected with the filaria as these affections are observed in natives who do not suffer from onchocerca nodules. He demonstrated the caecutiens microfilariae 'in great abundance' in pieces of skin taken from the ear and scalp of a caecutiens carrier.

Castellani (1925) is inclined on clinical grounds to believe that *O. volvulus* and *O. caecutiens* are not identical.

Blacklock (1927) is, on the other hand, inclined to believe that *O. volvulus* and *O. caecutiens* are the same.

The writer thinks there may have been some grounds for Brumpt and his supporters in creating and maintaining this new species before the discovery of the insect vector of *O. volvulus* by Blacklock, but since the demonstration of the complete life-cycle of the parasite outside the human body, there is no justification for this differentiation. This is especially so after the demonstration by Blacklock of the bionomics of the insect vector, *Simulium*, and his enquiries about the differences in the conditions of life and surroundings in the African and American endemic zones. In America the life-cycle in *Simulium* was worked out by Hoffmann in Mexico, who reported that the embryos of *O. caecutiens* in that country developed normally in *Simulium mooseri* and, besides, there is much evidence that this insect may prove to be the intermediate host in the other American endemic centres. Robles, the discoverer of *O. caecutiens*, has shown, after careful investigation, that only two species of fly both of the genus *Simulium*, *S. samboni* and *S. denelli* are found in the infected zone in Guatemala and, further, that

where these insects are most numerous there were also most cases of the disease.

According to Blacklock, the simulium does not attack man in bright sunlight as during such periods of the day it seeks shelter in shaded places such as in grass. It attacks man when the sun is low. He made a very important observation of a village in the Konno District, with a population of sixty-six; thirty-three, or 50 per cent., of these presented nodules, and no less than eight, or 24 per cent., had them on the head. He explained this high incidence by noticing that the village consisted of eleven houses and occupied a much smaller space than any other village of the same number of houses; the houses were arranged in a close ring, practically touching each other. He also noticed that the village is shaded by large trees, as is also the path down to the river beside which the village is situated. In such a place, the simulium, he said, can attack the upper parts of the body without exposing itself to the sunlight, hence the high percentage of tumours on the head.

He also made enquiries about the conditions in the coffee plantations in Guatemala, and was informed that coffee is most commonly grown under overhead shade. He concluded that this fact, plus the wearing of trousers and some kind of footwear, might explain the incidence of the tumours of this species on the head in Guatemala.

The writer would suggest that with such overhead shades in the coffee plantations it is possible that the simulium, instead of resting on the grass or plants on the ground, may rest on the ceiling or the under surface of objects giving this overhead shade and then when a human victim is passing below, the fly comes down for a feed of blood on the nearest part of the body, which is the head. Moreover, Rodhain (1920), in the Belgian Congo, came across a child bearing a cyst on the occiput: there occurred in addition some slight swelling of the face, puffiness of the features and dryness of the hair, which entirely disappeared when the cyst was removed. Thus the picture presented in this case bore great resemblance to coast erysipelas which is associated with caecutiens infections.

After these proofs and the work of Fülleborn in which he was not able to detect any morphological differences in the two species, there appears to be no necessity for upholding *O. caecutiens* as a separate species.

The writer believes that *O. volvulus* and *O. caecutiens* are one species and also that the coast erysipelas and the eye symptoms which are frequently associated with *O. caecutiens* are due to the presence of the worms and the embryos, being the result of a direct tissue reaction against the toxic products of these. Therefore the writer disagrees with Fülleborn, who is inclined to believe that these symptoms are not related to caecutiens infections. There is evidence of the fact that these eye symptoms and coast erysipelas occur only in the endemic areas of caecutiens onchocerciasis in tropical America, and are not reported in localities there where *O. caecutiens* is unknown. Thus, they have been reported in Guatemala and also by Fülleborn, in Mexico, where *O. caecutiens* according to him is endemic, and lately Lasumbe and Hardwicke (1928) reported *O. caecutiens* infections in Oaxaca, in Mexico, with eye symptoms which are relieved by incision of the nodules and evacuation of their contents.

Here the writer will attempt to explain the prevalence of the eye symptoms and the coast erysipelas from a pathological point of view, and show that it is only a matter of regional situation in the body that makes them peculiar lesions associated with onchocercal infection.

It is well known that the skin of the face is more delicate and softer and also richer in lymphatics than the rest of the skin surface of the body and is therefore more vulnerable to toxic and other irritants. The sting of an ant or a wasp which will produce little or moderate reaction if inflicted on the leg or arm, produces marked or severe reaction if inflicted on the face, attended with much swelling and oedema, puffiness of the eyelids and injection of the eyes, a picture very similar to an acute attack of erysipelas of the coast. To take an example from the helminthic infections themselves, in acute attacks of *Trichinella* and also in marked *Ancylostoma* infections, amongst the clinical signs and symptoms produced by the toxins of these helminths, oedema of the face and eyelids is very common without the appearance of oedema in the other skin regions of the body. In very heavy infestations with these worms, however, oedema of other regions may develop, as the feet and around the ankles, but long before this a marked oedema of the face will be present. This shows that the threshold of the toxin concentration in the blood is low in producing oedema of the face and has to be raised to produce oedema

of the other skin regions of the body. In this last example the worms are not in the face but in the muscles and intestines respectively, and their toxins only reach the face via the circulation.

Therefore, the factors responsible for the production of the eye symptoms and coast erysipelas are exactly the same as the factors working in the production of the localised thickened areas and other skin lesions described under *O. volvulus*. Thus in *O. caecutiens* the female worms, whether free and wandering in the skin structures of the head and face or lying alive inside the tumours, discharge from time to time large batches of embryos which swarm in the tissue lymph spaces of the face and scalp. This is supported by the above mentioned observation of Fülleborn who demonstrated the embryos ' in greatest abundance ' in pieces of skin taken from the ear and scalp of a caecutiens carrier. At the same time they secrete their toxins which accumulate in the tissue lymph and reach a certain concentration sufficient to irritate the local tissues to react in the same way as described under *O. volvulus* with the result that a small amount of fibrosis and thickening of the skin is left after each attack. But while the toxins are in their maximum concentration they injure the endothelium of the capillary blood vessels in the area with the result that a large amount of lymph transudes out of these vessels into the tissue spaces ; this transuded lymph fulfils many functions. These are the dilution of the toxin secreted by the embryos locally, the removal of some of the toxins away from the area to the general circulation via the lymphatic vessels of the part and also the supply locally of more nutrition to the cells of the part which are endangered by the toxins. This process of lymph transudation occurs also in *O. volvulus* skin lesions but the parts affected are not so soft and delicate as in the face. Marked oedema of the forehead and face thus occurs during the acute attacks and the cellularity of the tissues here allows more fluid to accumulate in the skin and subcutaneous tissues. In this way we can understand how these erysipelas-like attacks of the face are due to and associated with *O. caecutiens* infestations.

Manson-Bahr stated that these attacks recur every fifteen days and this the writer believes may be due to the periodic discharging of new batches of embryos from the parent worms. Every attack leaves behind it a certain amount of fibrosis with the result that in

the chronic and well-marked cases the skin of the face exhibits those elephantoid or myxoedematous appearances which are described by some authors.

The eye symptoms are due to the free anastomosis which exists between the lymphatics of the face and those of the eye; as this part is very richly supplied by lymphatics some of the toxin which is absorbed by the lymphatics goes to the eye, irritates it and produces the keratitis and other eye affections. These eye symptoms would continue with periods of exacerbation so long as the female worms are living and discharge embryos. The cornea, although a nonvascular membrane, is very rich in lymph spaces or channels which communicate freely with one another and with the lymphatics at its periphery so that the toxins can find their way easily to it and irritate it. There is also the probability that the embryos or even the parent females may be present locally in the tissues of the eyes and so their toxins irritate these directly. This is supported by the recent observations of Ochoterina (1930) in Mexico, who found numerous embryos of *O. caecutiens* in the excised eye of a blind man. On serial sections, the embryos were aggregated particularly 'in the outer third of the corneal epithelium.' This observation, further, supports the idea that the habitat of the embryos is in the tissue lymph spaces, because the cornea is a nonvascular membrane and formed by the corneal corpuscles bathed in the tissue lymph. When the tumours are removed with the worms in them the main source of the toxin is done away with and so the eye lesions improve if no permanent damage is done.

Fülleborn in his argument against coast erysipelas and the eye symptoms being due to *O. caecutiens*, stated that there are some natives who develop these lesions without exhibiting tumours. From what has been said about *O. volvulus*, this is easily explained by the fact that in such patients the worms are there but they are leading a free life in the tissues, discharging their embryos in the usual way without necessarily being encysted in palpable tumours.

Note.—While this paper was in the press an article by Professor Richard P. Strong in *Science* of May 29, 1931, was brought to my attention. In this it is recorded that the Harvard Expedition on onchocerciasis in Guatemala, had proved the development of the worm *O. caecutiens* in three species of *Eusimulium* probably *avidum* Hoffmann, *ochraceum* Walker and *mooseri* Dampff.

COMPARISON BETWEEN THE TUMOURS DUE TO *ONCHOCERCA VOLVULUS* AND *ONCHOCERCA GIBSONI*

So far as my material taken together with the descriptions in the literature permits, I shall endeavour to form a picture of the pathological effects. It must be noted that considerably less work appears to have been done in the case of *O. gibsoni* than *O. volvulus* as regards the description of the appearance in the worm area inside the capsule of the nodule.

(1) *The capsule*

In the material at the writer's disposal it was found that the capsule of the gibsoni tumour is distinctly thicker than that of the volvulus tumour. The thickness of the capsule was nearly equal to the diameter of the worm area itself. In the volvulus tumour the capsule was very thin and its fibres were very compactly pressed together. The Harvard Expedition to Liberia found a large volvulus tumour whose capsule was macroscopically about 2 mm. thick, but they did not mention anything about its histology.

De Kock commented on the thickness of the capsule of the gibsoni tumours and divided the gibsoni nodules into two groups according to their position in the subcutaneous connective tissues, viz. :— (1) very superficially placed nodules that can hardly be said to be situated in the subcutaneous tissues and which are situated on the surface like buttons, and (2) those which appear as swellings in the subcutaneous connective tissue revealing their presence by slight bulging of the tissue. Also occasional nodules which are detected in the intramuscular connective tissue of the deep pectoral muscle.

The superficial nodules, he said, show a thin fibrous capsule, enclosing a well defined nucleus or worm area which in the majority of cases measures about 1 mm. in thickness. The deep-seated tumours on the other hand are found to have a much thicker capsule enclosing a well-defined worm-nest. On measurement he found that although the deep-seated tumours are much larger than the superficial ones they do not enclose a larger worm nucleus, e.g., a tumour 3·9 cm. in diameter has a worm area of only 0·9 cm. in diameter, while on one side the capsule was 1·3 cm. and on the other 1·7 cm. thick. He is, therefore, of opinion that the thickness of the capsule largely

depends on the situation and that the size of the worm-nest has no relation to the thickness of the capsule around it.

The tumour which the writer examined was removed from the brisket of an animal and so according to De Kock's classification would fall in the deep-seated tumours with thick capsules. Further, it was noticed microscopically that the capsule of the gibsoni tumour was more cellular especially towards its inner layers where the embryos were also numerous, while the volvulus capsule was made up of dense fibrous tissue with fewer cells and no embryos in it. Again the blood vessels of the gibsoni capsule, especially in its outer layers were very much thickened or even completely obliterated and often surrounded by a cuff of lymphocytes with plasma cells, while the blood vessels of the volvulus capsule looked quite normal with the exception of some vessels in the areolar connective tissue around the capsule which showed a perivascular infiltration with plasma cells, lymphocytes and a slight thickening of their walls.

These differences in the capsules of the two tumours cannot be explained. But the greater thickness and cellularity of the gibsoni capsule is due to the presence of a large number of embryos which kept up a continuous subacute productive inflammatory process going on especially in its inner layer. This cause may account also for the thickness of its vessels ; but why it is that the capsule of the volvulus tumour is not similarly heavily invaded with embryos and does not show a similar histological picture, one cannot say.

De Kock stated that the superficial nodules had thin capsules, but he did not refer to their histology. Can we say that these superficial tumours have thin capsules which may be similar in structure to the capsule of the volvulus tumours, because in these superficially placed tumours and those of *O. volvulus* as well, the embryos which are discharged from the females inside make their way out through the capsule to the lymphatic spaces of the skin around the tumour ? Skin being their natural habitat they do not stay in the capsule long enough to stimulate it to react ; in the deep-seated tumours, the embryos finding that the tissue spaces of the muscles around are not suitable for their life, stay in the capsule irritating it and causing ultimately its greater thickness and cellularity.

(2) *The Worm Area.*

The worm area of *O. gibsoni* tumours is very small compared with the size of the tumour, the bulk of the tumour being due to the thick capsule. Moreover, the worm sections it contained, even if we compare it area for area with the volvulus tumour, were much fewer than in the latter.

The matrix of the gibsoni tumour was mainly made of hyaline fibrous tissue with comparatively fewer cells and practically no cellular reaction comparable to that seen in its capsule, except around some of the worm sections which have been described under the histology of this tumour. The matrix of the volvulus tumour, on the other hand was on the whole very cellular showing a great deal of tissue reaction both in the stroma and also around a fair number of the worm sections.

These differences in the worm nuclei of the tumours could be explained by the fact that the volvulus tumour contained a larger number of worms than the gibsoni tumour and that there were greater numbers of embryos being discharged from the volvulus worms, and that these kept up the reaction in the stroma.

According to Gilruth and Sweet, the gibsoni tumours appear to contain most generally one female worm only and less commonly one male and one female. On the other hand, Manson-Bahr stated that the volvulus tumour contains at least four male and two female worms. This may explain the small size of the nuclei of the gibsoni tumours compared with the large-sized nuclei of the volvulus tumours.

The various workers on the gibsoni tumours reported the frequent occurrence of degenerative changes and calcification in the worms inside the tumours, while such degenerative phenomena are hardly ever reported in the literature of the volvulus tumours. This difference, however, may be only apparent and may be due to the fact that the workers on gibsoni had more opportunities to examine large numbers of tumours removed than the workers on the human onchocerca.

THE EOSINOPHILE CELL IN HELMINTHIC INFECTIONS

It may not be out of place here to say a few words about the eosinophile cell because eosinophilia either local in the tissue or general in the circulation is a characteristic of helminthic infections. This cell is a true haemocyte and is present in the blood normally forming from 2 to 4 per cent. of the total leucocytic count. When stained with eosin it shows brilliantly coloured pink granules in the protoplasm ; these granules are larger and much more refractile than those of the polymorphonuclear neutrophile leucocyte. The eosinophiles are very fragile and readily break down, discharging their granules. They do not appear to play any very important part in general or specific bacterial inflammatory conditions. In diseases due to animal parasites on the other hand, they may be very markedly increased in the blood and may form large collections in the tissues in and around inflammatory foci excited by these parasites. Eosinophilic leucocytosis is found also in asthma, eczema, pemphigus, urticaria and other skin diseases, in cases of anaphylaxis and in certain infectious diseases, especially scarlet fever and when an echinococcus cyst ruptures in the abdomen. Histoeosinophilia or collections of eosinophiles in the tissues occurs in and around helminthic deposits, in Hodgkin's disease and around the bronchi in asthma.

That the helminths and their body extracts contain eosinophilotactic substances which stimulate the mobilisation of the eosinophiles is shown by the work of Vallillo, Panizza, Herrick, Weinberg, and Séguin. Some of these workers have been able to produce general or local eosinophilia by the injection of extracts of the parasites or have been able to attract the eosinophile leucocytes into capillary tubes, containing extracts and placed in the tissues. Botteri (1925) found after a twelve-hour positive intradermal test for hydatid disease an intense local eosinophilia. Regarding the functions of the eosinophile cells in helminthic infections nothing much has been said. Sorour (1930) stated that he often came across organs and tissues in which bilharziasis and tuberculosis existed so close to each other as actually to appear side by side in the same microscopic field. In these cases he was always impressed by the dense and perfect fibrotic encapsulation of such tubercular lesions,

as if to suggest that the conditions created by the bilharzial factor favoured the fibrous healing process of the neighbouring tubercular lesions.

In order to find an explanation he tried to compare the tissue reactions excited by the bilharzia ova and by the tubercle bacillus in order to find out any elements of these reactions that may be present in bilharzia and which may stimulate fibrosis and be absent in tuberculosis. For this purpose he analysed the lesions produced by bilharzia and divided them into two, viz. :—(1) Superficial or open lesions where the bilharzial deposits occur above the muscularis mucosa of the hollow organs such as the bladder and intestine. In these the ova are deposited in a loose vascular cellular tissue, the cells present being endothelial and plasma cells without eosinophiles and the covering epithelium is partially destroyed by bacterial infection so that the ova can pass out of the body to propagate the species. (2) Deep or closed lesions below the muscularis mucosa in the hollow organs or in solid organs such as the liver, spleen, glands, etc., where the ova are destined to be shut in a strong capsule of fibrous tissue. In such deep lesions the ova could produce two types of tissue reaction and in this capacity the analogy with the tubercle bacillus is perfect. The first type of tissue reaction is a diffuse cellular moderately vascular tissue with bilharzia ova scattered in it, surrounded or not by giant cells: the cellular elements consist in the earlier stages of plasma cells and endothelioid cells. Later on eosinophiles begin to appear among the above-mentioned cells. This last event, he states, is a very important one, because repeated observations have convinced him that the appearance of the eosinophiles is always contemporary with the commencement of a fibrous transformation of the recent bilharzial cellular reaction tissue. This fibrous transformation continues till all the lesions are densely fibrosed, by which time the causative ova may have all died and be calcified if not destroyed by the once present giant cells. Then the eosinophiles begin to disappear so that they may be entirely absent in chronic fibrosed bilharzial lesions.

In the second type of tissue reaction are found *discrete* minute bilharziomata embedded in a fibrous matrix. The histology of these is very interesting on account of the great analogy with the miliary tubercles. Thus each consists of a central ovum surrounded by

large giant cells and then by many layers of typical endothelial cells forming an avascular nodule which when present on the pleura or peritoneum looks like miliary tubercles; he therefore named these ' miliary bilharziomata.'

Later on eosinophiles begin to appear heavily around these miliary bilharziomata, i.e., in the fibrous matrix in which they are embedded and again its appearance is always contemporary with the commencement of a hyaline fibroid change in the peripheral layers of the endothelial cells. This fibroid change continues concentrically inwards until all the miliary bilharzioma is transformed into a mass of hyaline scar similar to that seen around healing tubercular lesions, Here, again, as the process of fibroid transformation is completed, the eosinophiles disappear while the central ova die and calcify if they are not destroyed by giant cells.

From the above-mentioned facts he concluded it justifiable to attribute to the eosinophile cell the stimulus to the commencement and completion of the fibroid transformation of the endothelial elements in the bilharzial reaction tissue. He next surveyed the histological changes of other diseases to find out if the eosinophile played the same part in the fibrosis of the endothelial reaction tissue in other lesions besides bilharziasis. He found they played the same rôle in two other diseases, namely, chronic filarial funiculitis and Hodgkin's disease. Thus he found that the filarial toxins produce endothelioid proliferation and giant cells and cause locally heavy accumulation of eosinophiles; here also the end result was a dense fibrotic lesion. Again, in Hodgkin's disease, the tissue reaction starts by endothelioid cell proliferation and the formation of giant cells; the eosinophiles form also another element of the tissue reaction in that disease which nearly always ends by fibrosis in the later stages of the disease. He then formed a table of four diseases, namely, (1) bilharziasis, (2) chronic filarial funiculitis, (3) Hodgkin's disease, and (4) tuberculosis. In all these are endothelioid cells and giant cells; in the first three diseases there are eosinophiles and they end in fibrosis; in tuberculosis there are no eosinophiles and it often ends in caseation.

He now supported this alleged function of the eosinophile cell by stating that (1) tuberculosis, which is very common in all the organs and tissues in Egypt, is, however, very rare in the urinary tract and

this is due to the presence of bilharzia ova and worms in that tract, and that (2) he had collected the post-mortem statistics since 1905 and found that the association of tuberculosis and bilharzia was very rare as a cause of death ; (3) ancylostome in Egypt which causes high eosinophilia is rarely associated with tuberculosis in causing death, as seen from the above statistics also, and (4) that he treated two patients suffering from bilharzia with tartar emetic, and a few weeks after they were cured of bilharzia, they both developed active tuberculosis.

From the above data the reader can see that Sorour is much impressed by this new function which he attached to the eosinophile cells. Moreover, he is now experimenting on the effect of artificially produced eosinophilia on the healing of tuberculous lesions in experimental animals ; the results of his researches are not yet published. It should be remarked that in his comparison of the lesions produced by bilharzia and tuberculosis, he disregarded the presence of the lymphocyte and the rôle it plays in tubercular deposits and also disregarded the great potency of the toxin of the tubercle bacillus. It is admitted that the eosinophile cells are very often seen, sometimes in large numbers in and around bilharzial deposits, and that their appearance is contemporary with the commencement and completion of the fibroid transformation of the endothelial elements which appear around the ova immediately after their deposition The writer does not agree with Sorour on the function he attached to this cell in the process of fibrosis, and supports the workers mentioned later, who attribute to it a totally different function. The eosinophile cells are closely related to the polymorphs in their structure and behaviour and so their functions most probably are closely allied. The lymphocyte, too, in its behaviour has also many points of similarity to the two above-mentioned cells.

Thus in acute infections the polymorphonuclear cells appear in abundance in the blood forming a polymorphonuclear leucocytosis and they migrate in great numbers to the focus of infection. They combat the invading bacteria by ingesting and digesting them and by the secretion of various bactericidal and detoxicating substances including proteolytic and oxidising enzymes. There is very little doubt that these neutrophil cells do also produce proteolytic ferments capable of digesting fibrin in inflammatory exudates. It is quite

possible that these cells being granular, the secretions which they liberate are formed by the activity of the granules.

In every process of inflammation there is a struggle involving two processes between the body as a whole and the invading agent, whether bacterial or otherwise. (1) The killing of the invading germ with neutralisation of the toxic products discharged from it ; this is carried out by the polymorphonuclear cells in general inflammations. (2) The subsequent repair of any damage that may have been inflicted on the local tissue by the germs ; this is accomplished by proliferation of the fixed connective tissue cells and blood vessels of the area, ultimately ending in the formation of scar tissue. If the struggle between the bacteria and polymorphs takes a long period this process of fibrosis tends to build up a fibrotic capsule around the invaded area. But this process of fibrosis cannot be started and completed to the best advantage if the cells of the part, especially the connective tissue cells which initiate this process of fibrosis, are bathed in and poisoned by the toxins of the bacteria. These toxins must be neutralised first and the field cleared in order to give the process of fibrosis the chance to be carried out. So in acute inflammations the polymorphs ingest the bacteria and neutralize their toxins and when they succeed in doing this, the process of repair starts. An exactly comparable process occurs in helminthic and tubercular infections but in these diseases the part taken by the polymorphs as described above is taken by the eosinophiles in helminthic infections and by the lymphocytes in tubercular lesions. Thus when the body is invaded by a worm parasite the eosinophile cells immediately respond and we get a general eosinophilia and also a histoeosinophilia around the helminths if these are deposited in the tissues. The eosinophiles here have the same function towards the helminths and their toxins as the polymorphs towards the bacteria. They thus secrete antitoxic products which neutralise the toxins secreted by the worms or their embryos and perhaps also they secrete other products which are inimical to these parasites themselves. When the worms are lying in the hollow organs such as the intestines or the lumen of the vessels, their toxins are distributed equally in the blood and tissues and so we get a general eosinophilia. If on the other hand the worms or their progeny invade the tissues, then we get a local concentration of the toxic products in those parts of the

tissues, with a part of the toxin, diluted, and absorbed by the local lymphatics, ultimately reaching the circulation. Here also we get a general eosinophilia but the majority of the circulating eosinophiles are recruited from the blood to the foci of helminthic deposits in the tissues resulting in large accumulations of these around, e.g., the bilharzia ova. The deposited ova here with the toxins secreted by the embryos inside, excite the tissues to react around them in exactly the same way as when tubercle bacilli or any other organisms are deposited in the tissues. In other words we get an inflammatory reaction locally which manifests itself by the appearance of the endothelial, giant and plasma cells. These cellular elements plus the fixed connective tissue cells of the part which initiate the process of fibrosis are bathed in the toxic products of the worm, and so are unable to carry out their functions. The eosinophile cells appear then in large numbers in order to neutralise as much as possible all the toxic products in the area. In this way and with the appearance of these latter cells the endothelial cells and the fixed connective tissue cells are given the chance to start the process of fibrosis which shuts the ovum in a fibrous more or less avascular nodule. On the completion of fibrosis the bilharzia ovum with its embryo will have died and become calcified or phagocytised and absorbed by the giant cells, so that the source of toxins is done away with, and the eosinophiles having done their work, disappear. But so long as the worm or embryos are alive the eosinophiles will be present.

Thus the function of the eosinophile cell in helminthic infections is not that of directly stimulating fibrosis, but to neutralise the toxic principles which are diffused locally and which by poisoning the cells have prevented them from carrying out their proper reparative processes.

In cases of long continued helminthic infections as for example, in chronic and marked ancylostomiasis, where the haemopoetic tissues are exhausted and so the eosinophilic response falls, the toxins accumulate in the blood and produce the well-marked symptoms observed in such cases.

This function of the eosinophiles which the writer supports is emphasised in the work of many authors.

Huebner (1911) as a result of animal experiments, found that the eosinophiles accumulated around encysted trichina in the muscles to

attempt the removal of the toxins. The work of Weinberg and Séguin (1915) on the functions of the eosinophiles is rather important. They found that the oesinophiles would phagocyte certain bacteria, fungi, protozoa and inert particles such as carbon, both *in vitro* and *in vivo*. Moreover, they found that eosinophiles when mixed with hydatid fluid and incubated for one hour at 37° C., lost their power to phagocyte certain organisms and the cyst fluid lost its reactivity as a test antigen in complement fixation tests. This absorption power was manifested to a less extent by the polymorphs and was practically nil for the blood monocytes. They thus concluded that the eosinophile cells like the polymorphs play an important rôle in immunity, but while the principal function of the polymorphs is the protection of the organism against bacterial infection, the eosinophiles are adapted to the neutralisation of certain toxic substances. The eosinophiles can manifest their capacity for the phagocytosis in the absence of the polymorphs and the polymorphs can also absorb the toxic products of helminths in the absence of eosinophiles.

Taliaferro (1927) states that the eosinophiles are closely related to the other granular leucocytes especially the polymorphs. In function, recent work has shown the two to be somewhat similar except that in the eosinophiles there is less emphasis on phagocytosis and more on the detoxication of foreign materials. In fact, the eosinophiles are supposed to be practically concerned in the removal of foreign proteins or their split products. They therefore occur in great numbers in such conditions of protein hypersensitiveness, as anaphylaxis, serum sickness and bronchial asthma when they are found locally or generally, depending on the distribution of the foreign protein.

In tuberculosis the lymphocyte which Sorour had neglected has its own specialised function like the other granular leucocytes of the blood, the polymorphs and eosinophiles. It is a granular cell like the others containing in its protoplasm the so-called ' Altmann granules,' which are fuchsinophile in staining and play a rôle in tuberculosis exactly similar to that of the eosinophile cell in helminthic infections. Thus in the formation of a miliary tubercle we get at the beginning endothelioid cell proliferation and giant cells. Soon after the lymphocytes appear in great numbers and come to rest at the

periphery of the tubercle and between the epithelioid cells, and remain there so long as the tubercular process is active. In favourable cases as in fibroid tubercles where the tubercle is fibrosed they disappear. During the active stages of tuberculosis there is usually, besides the local accumulation of lymphocytes around the tubercle foci in the tissues, a general lymphocytosis in the blood as well.

Therefore, the writer is of opinion that the lymphocytes play an important rôle in the neutralisation of the tuberculin secreted by the tubercle bacillus and so help in an indirect way the process of fibrosis in or around the tubercular deposits by creating better conditions for the endothelioid and the fixed connective tissue cells to perform their functions towards fibrosis, thus killing or at least shutting off the tubercle bacilli and their toxins from the rest of the body.

When the virulence of the organisms is strong and their toxins potent, then the recruited lymphocytes are overwhelmed and cannot neutralise the whole tubercular toxin, with the result that the endothelioid cells of the miliary tubercle are poisoned and undergo necrosis and caseation which spreads outwards with little or no fibrosis around. If the tubercle bacilli are attenuated and their toxin weak, then the lymphocytes can destroy it all and so there will be no caseation of the endothelioid cells which ultimately become transformed into fibroid tissue. Thus the lymphocyte in this capacity seems to play a very important rôle in tuberculosis similar to that played by the eosinophiles in helminthic infections.

Lichtenstein (1921) supports this function and attributes to the lymphocyte a bactericidal capacity towards the tubercle bacillus and a power probably, by means of a lipase, to destroy its acid fastness, its general virulence and its caseating power.

Thus, the eosinophiles have an undoubted function in relation to helminthic and some other infections, a function which the writer has endeavoured to elucidate here.

SUMMARY AND CONCLUSIONS

1. A historical review of the pathological manifestations caused by human and some bovine onchocercal infestations is made; the descriptions given of the histological changes excited in the tumours show great variation.

2. The writer examined an *O. volvulus* and an *O. gibsoni* tumour and found, especially in the volvulus tumour, that the tunnel walls and the stroma between them were made up of either purely cellular tissue, the cells being mainly endothelioid, or of immature fibrous tissue, or else of adult fibrous tissue with fewer cells, these being mainly adult connective tissue cells.

3. It is suggested that in the formation of the tumours, the whole body of the worm does not encyst all at one time, but segment by segment, each producing around itself a reaction, beginning with endothelioid cells and ending in mature fibrous tissue which forms the worm canal for the segment. This process is repeated until the whole length of the body of the worm is dragged into and encysted inside the tumour.

4. The varying histological pictures in the tunnel walls of the tumours are explained on the above theory, the cellular areas in the stroma being the result of a reaction against the concentrated toxins locally of the worms and embryos; this cellular tissue later becomes fibro-cellular and ultimately adult fibrous tissue.

5. It is quite possible that juxta-articular nodules in the endemic volvulus centres may be old volvulus tumours in which the worms have died and are absorbed or liquefied.

6. Evidence is produced which shows that the tissue reactions excited by onchocerca are similar to those excited by *Wuchereria bancrofti*, the schistosomes and probably other helminths invading the tissues.

7. The tissue changes caused by helminthic deposits show a great similarity to those presented by some of the infective granulomata, both in their histology and also macroscopically by forming tumour-like masses.

8. There is little doubt that the helminthic parasites do secrete toxins which are responsible for the general and local symptoms complained of by parasitised patients.

9. The lesions, other than the tumours, caused by *O. volvulus* such as localised thickenings of the skin, elephantiasis and enlarged glands are most probably due to the local concentration of the toxins in hyperinfections with embryos, resulting in repeated tissue reactions which end in fibrosis and ultimately destroy the skin structures and obliterate its lymphatic network.

10. The eye symptoms and erysipelas of the coast which are associated with *O. volvulus* infection in South America are due to local concentration of the toxins secreted by the worm and embryos just as in Africa. The affection of the head in South America is explicable on the bionomics of the vector.

11. The histological picture seen in the *O. volvulus* and *O. gibsoni* tumours examined by the writer showed some differences.

12. It is argued from the histological appearances that the eosinophile cell serves the function of neutralising the toxins secreted by the helminthic parasites.

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THE ACTION OF VARIOUS SERA, *IN VITRO*, ON THE GUT AND SALIVARY GLAND FORMS OF *T. RHODESIENSE* AND *T. GAMBIENSE* FROM *GLOSSINA PALPALIS*

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During the course of an investigation into the action, *in vitro*, of various sera on a number of strains of *T. rhodesiense* and *T. gambiense* in laboratory animals, the opportunity was taken of determining the reactions to these sera of the various stages of the parasites occurring in experimentally infected *Glossina*. The only species of fly readily obtainable in sufficient quantities, locally, for work of this nature, is *G. palpalis* and it is with this species that the following experimental work has been performed. Reference to the literature available reveals little work on the serological reactions of the development forms of the pathogenic trypanosomes. Johnson and Lloyd (1928) found that the fresh sera of a number of animals were actively trypanolytic to the developmental forms of *T. congolense* and that fresh sheep serum was the least trypanolytic of those tested. They found that drying sera on a filter paper and storing for over a year caused a loss of trypanolytic power, and that in blood serum deactivated to 65° C. for thirty minutes and suitably diluted with Ponselle's medium, the trypanosomes multiplied rapidly up to the eighth day. They note that ' bacterial infection rapidly destroys the developmental forms of *T. congolense* whether *in vitro* or *in vivo*.'

The flies used in the following work were kindly supplied by Dr. H. L. Duke, from the various transmission experiments in which he is at present engaged. They were hatched out in the laboratory from pupae collected in the field, and were given, after hatching, one or two feeds on laboratory animals infected with the strain

under consideration, being subsequently kept alive by feeding on clean laboratory animals. On about the twenty-fifth day from the last infecting feed, the flies were chloroformed and dissected by Dr. Duke, by the posterior method employed at this laboratory. The gut and salivary glands were removed into Ringer-glucose solution, and those organs found to contain trypanosomes were dealt with in a manner subsequently detailed.

The strains of trypanosomes used were :—

(1) Four strains of *T. rhodesiense*, known as ' A,' ' B,' ' C,' and ' D,' respectively (Laboratory strains XLIII, XLIV, XLV and XLVI), all very recently recovered from human cases in the Kahama area of Tanganyika. The majority of the flies used were infected either from the original guinea-pigs inoculated from man or from monkeys subinoculated from these guinea-pigs.

(2) A strain of *T. gambiense*, ' Yembuga ' (Laboratory strain XXXIII), isolated from man in the West Nile area of Uganda early in 1929, and since maintained in a sheep and several monkeys by cyclical transmission through *G. palpalis*.

TECHNIQUE

The infected gut or gland was thoroughly broken up with needles in about 0·25 c.c. of Ringer-glucose solution, and the resultant suspension of parasites and body tissue was evenly distributed among two or three small sterile glass tubes. These tubes, already described in a previous publication (Yorke, Adams and Murgatroyd, 1929), are made from glass tubing about 7·5 cms. in length and 7·5 mm. in bore, and are covered with glass caps made from tubing of a slightly larger diameter. To each tube was then added a sample of the serum to be tested, the volume added being such as to give a concentration of serum of about one part in two in each tube. The contents were shaken thoroughly, an initial count of the trypanosomes was made from one tube on a Thoma-Zeiss haemocytometer slide, and the tubes were then placed in a shady place at laboratory temperature (24° to 29° C.), and re-examined at intervals.

Experiments were first undertaken with the object of finding a medium capable of maintaining the trypanosomes alive *in vitro* for a suitable period, preferably about twenty-four hours, and to this end

the sera were freshly obtained for each experiment, it having been found inadvisable, for a variety of reasons, to use sera more than one or two days old. Owing to the impossibility, in work of this nature, of sterile dissection of the very large numbers of flies necessarily examined, bacterial contamination frequently occurred in the tubes. In spite of the considerable bacterial growth often present at the end of twenty-four hours, those trypanosomes still living usually presented an apparently normal appearance and were vigorously active. Accurate counting of the parasites was sometimes a matter of some difficulty owing to their tendency at times to clump together; these clumps are exceedingly difficult to disrupt, and the trypanosomes forming them often appear to be undergoing a degenerative process. In Table I are recorded a number of observations made on a variety of sera, fresh and heated, undertaken to find a suitable medium for the maintenance of the gut-forms of *T. rhodesiense in vitro.*

Study of Table I reveals the fact that all the fresh sera employed were rapidly toxic to the gut forms of the strains of *T. rhodesiense* examined, whereas heating these sera to 62° C. for a period of twenty minutes destroyed the trypanocidal body in each of them, and in a number of cases there is definite evidence of multiplication of the trypanosomes in the heated sera. The observations were confirmed in each case by examination of numerous flies and in every case the fresh sera rapidly destroyed the gut forms of *T. rhodesiense,* while in heated sera the parasites survived for a considerable period. It thus appeared that sera of a variety of animals if heated to 62° C. for twenty minutes would support the gut forms for a sufficient period for their study *in vitro* at room temperature (24° to 29° C.). In order to investigate further the nature of the trypanocidal body in the unheated sera, a number of them were heated to different temperatures for twenty minutes and in Table II are set forth the results of experiments of this nature.

Examination of Table II shows that whereas all the fresh sera investigated are actively trypanocidal, heating them to 48° C. for twenty minutes does not destroy their trypanocidal powers, while heating them to 56° C. or to 64° C. for the same time does do so. As heating to 56° C. for this period removes haemolytic complement, it appeared that this component might be the active body. Examination of the sera for complement after the experiments

TABLE I

Showing the period of survival, *in vitro* at room temperature, of the gut forms of *T. rhodesiense* from *G. palpalis* in a variety of sera, fresh and heated to 62° C. for 20 minutes.

Fly	Strain	Serum	Treatment of serum	Number of living trypanosomes per 256 squares of the haemocytometer scale					
				Start	1 hour	2 hours	4 hours	6 hours	24 hours
1	D	Rabbit ...	Unheated	42	0	...	...	...	...
			62° C. for 20 minutes	...	44	...	50	...	32
2	C	Guinea-pig ...	Unheated	34	0	...	...	...	...
			62° C. for 20 minutes	...	46	...	52	...	54
3	C	Sheep... ...	Unheated	42	0	0	...	...	...
			62° C. for 20 minutes	...	38	34	...	36	38
4	D	Monkey ... (*Cercopithecus* sp.)	Unheated	55	...	0	...	...	...
			62° C. for 20 minutes	...	...	48	...	...	94
5	D	Goat	Unheated	36	2	0	...	...	...
			62° C. for 20 minutes	...	38	44	...	...	64
6	D	Fowl	Unheated	42	0	...	0	...	...
			62° C. for 20 minutes	...	36	...	38	...	29
7	A	Crocodile ...	Unheated	28	0	...	...	...	...
			62° C. for 20 minutes	...	58	...	64	...	86
8	D	*Varanus* ...	Unheated	42	0	...	...	...	...
			62° C. for 20 minutes	...	34	36	38	34	98
9	D	Baboon ...	Unheated	38	2	0	...	...	...
			62° C. for 20 minutes	...	26	24	...	19	18
10	C	Human ...	Unheated	85	5	0	...	...	...
			62° C. for 20 minutes	...	98	104	96	110	94

TABLE II

Showing the effect of sera heated to different temperatures for 20 minutes on the gut forms of *T. rhodesiense*, *in vitro* at room temperature.

Fly	Strain	Serum	Treatment of serum	Number of living trypanosomes per 256 squares of the haemocytometer scale					
				Start	1 hour	2 hours	4 hours	6 hours	24 hours
1	A	Rabbit ...	Unheated	...	0	...	...	...	...
			48° C. for 20 minutes	...	0	...	...	...	...
			56° C. for 20 minutes	28	24	19	21	...	35
			64° C. for 20 minutes	...	20	18	...	25	34
2	A	Sheep... ...	Unheated	24	1	0	...	...	...
			48° C. for 20 minutes	...	0	0	...	...	...
			56° C. for 20 minutes	...	24	20	...	18	15
			64° C. for 20 minutes	...	22	32	...	16	13
3	D	Monkey ...	Unheated	55	0	...	0	...	...
			48° C. for 20 minutes	...	2	...	0	...	...
			56° C. for 20 minutes	...	39	...	48	...	96
			64° C. for 20 minutes	...	47	...	...	54	92
4	D	Goat	Unheated	36	...	0	0	...	...
			56° C. for 20 minutes	...	...	48	46	...	52
			64° C. for 20 minutes	...	...	42	36	...	68
5	A	Fowl	Unheated	36	...	2	0	...	...
			56° C. for 20 minutes	...	...	28	32	...	42
			64° C. for 20 minutes	...	...	52	54	...	64
6	A	Crocodile ...	Unheated	30	0	...	...	...	...
			56° C. for 20 minutes	...	36	42	48	...	20
			64° C. for 20 minutes	...	40	54	57	...	34
7	D	Baboon ...	Unheated	36	0	...	...	...	...
			48° C. for 20 minutes	...	2	2	...	0	...
			56° C. for 20 minutes	...	25	29	...	35	33
			64° C. for 20 minutes	...	...	34	...	...	27
8	C	Human ...	Unheated	85	5	0	...	...	...
			56° C. for 20 minutes	...	89	98	94	110	42
			64° C. for 20 minutes	...	102	94	104	96	88

revealed its presence in the fresh sera and in those heated to 48° C., and its absence from those heated to 56° C. and to 64° C. In order to remove complement without at the same time abstracting any antibodies present, a number of sera were filtered through a Berkefeld filter candle, as described by Muir and Browning (1909), and the experiments repeated. The results of some experiments of this type are recorded in Table III.

Reference to Table III lends support to the inference that complement is indeed the agent lethal to the gut forms of *T. rhodesiense.* Samples of the sera used were tested for the presence of complement, and none was detected in those filtered or heated to 62° C. for twenty minutes. In each case the filtered sera maintained the parasites alive for a considerable period, although in several instances the number remaining alive at the end of twenty-four hours was exceedingly low. This may be explained by the extraordinarily profuse growth of bacteria present in the majority of the tubes containing filtered sera after this period, but it is clear from the Table that whereas the fresh sera were all very rapidly lethal, the trypanosomes survived in the filtered sera for comparatively long periods undamaged.

In order more fully to demonstrate that the complement, present in varying degrees, in normal sera is toxic to the gut forms of *T. rhodesiense* from *G. palpalis* a number of sera, in which the complement had been destroyed by treatment with ammonia, were next examined. The process of inactivation employed was that described by Gordon, Whitehead and Wormall (1926), and was performed as follows :—to 4 volumes of serum was added 1 volume of N/6 NH_4OH, and the mixture was incubated at 37° C. for one and a half hours, after which N/8 HCl was added to adjust the reaction to pH 7·5 ; the control sera were diluted to a corresponding extent with Ringer-glucose solution. As a result of this dilution the concentration of serum in each tube of the experiment was about 1 in 4 or 1 in 3, that is about half to two-thirds that of the experiments already recorded in the preceding Tables. Table IV shows some typical results of experiments conducted with sera so treated.

From this Table it will be seen that deactivation of the sera with ammonia also removed the trypanolytic body. The longevity of the trypanosomes in the ammonia treated sera was not great, but this

TABLE III

Showing the effect of filtration through a Berkefeld filter candle on the trypanolytic action of various sera on the gut forms of *T. rhodesiense*, *in vitro* at room temperature.

Fly	Strain	Serum	Treatment of serum	Number of living trypanosomes per 256 squares of the haemocytometer scale					
				Start	1 hour	2 hours	4 hours	6 hours	24 hours
1	D	Rabbit ...	Untreated	26	0	...	...	...	...
			Filtered	...	30	...	42	...	24
			62° C. for 20 minutes	...	...	...	...	..	28
2	C	Guinea-pig ...	Untreated	34	0	...	...	...	...
			Filtered	...	36	...	35	...	4*
			62° C. for 20 minutes	...	46	...	52	...	54
3	B	Sheep... ...	Untreated	30	0	...	...	...	...
			Filtered	...	20	...	14	...	2*
			62° C. for 20 minutes	...	24	...	19	...	21
4	D	Goat	Untreated	36	2	...	0	...	...
			Filtered	...	42	...	38	...	40
			62° C. for 20 minutes	...	...	...	36	...	64
5	C	Fowl	Untreated	62	4	0	0	...	...
			Filtered	...	52	...	34	28	2*
			62° C. for 20 minutes	...	38	...	40	.	12*
6	A	Crocodile ...	Untreated	18	0	...	...	...	...
			Filtered	...	24	...	20	26	0*
			62° C. for 20 minutes	...	64	...	60	..	85
7	B	Baboon ...	Untreated	32	0	...	...	...	...
			Filtered	...	34	...	26	...	14*
			62° C. for 20 minutes	...	26	...	24	...	13*
8	A	Human ...	Untreated	20	0	...	...	...	...
			Filtered	...	22	22	24	19	0*
			62° C. for 20 minutes	...	24	17	25	22	22

* Signifies an unusually heavy bacterial growth.

may be explained by the unusual salt concentration in these media, and the possible adverse effects of the ammonia added. It is at all events clear that the trypanocidal action does not occur rapidly as in untreated sera. In other experiments with these same sera, similar results were obtained, and the data published are representative of a number of experiments with the different strains.

TABLE IV

Showing the effect of treatment with ammonia on the trypanolytic action of various sera on the gut-forms of *T. rhodesiense*, *in vitro* at room temperature.

Fly	Strain	Serum	Treatment of serum	Number of living trypanosomes per 256 squares of the haemocytometer scale					
				Start	1 hour	2 hours	4 hours	6 hours	24 hours
1	A	Rabbit ...	Unheated	58	0	...	...	...	...
			NH_4OH	...	58	52	...	46	0
			56° C. for 20 minutes	...	82	74	...	52	43
2	C	Guinea-pig ...	Unheated	34	0	...	...	...	...
			NH_4OH	...	46	...	54	...	8
			56° C. for 20 minutes	...	42	...	44	...	47
3	A	Sheep... ...	Unheated	54	0	...	...	...	...
			NH_4OH	...	38	...	28	...	0
			56° C. for 20 minutes	...	48	...	32	...	26

In view of these facts it became of considerable interest to examine the action of these sera on the gland forms of the strains investigated. Unfortunately, owing to the relatively low number of flies with heavily infected glands, and also to the small bulk of trypanosomes present in even a heavily infected gland, the concentration of parasites obtainable was usually very low. The results recorded in Table V, however, are sufficiently sharp to permit of no doubt as to the action of the sera employed.

It will be seen from Table V that fresh sera have no destructive action on the gland forms, and that they survive as well in fresh as in deactivated sera, thus differing from the gut forms of the same strains. Were this not the case it would be difficult to explain how infection of a susceptible animal occurred by injection of these forms into its subcutaneous tissues. From these experiments it will

thus be seen that whereas the gut forms are rapidly destroyed by fresh sera, in which the toxic agent is complement, the gland forms are completely resistant to the action of complement *in vitro* over the period of observation. Attention was next directed to the ' Yembuga ' strain of *T. gambiense,* and in Tables VI and VII are set

TABLE V

Showing the effect of a variety of fresh and deactivated sera on the gland forms of *T. rhodesiense* from *G. palpalis*, *in vitro* at room temperature.

Fly	Strain	Serum	Treatment of serum	Number of living trypanosomes per 256 squares of the haemocytometer scale					
				Start	1 hour	2 hours	4 hours	6 hours	24 hours
1	D	Goat	Unheated	3	...	2	...	3	4
			64° C. for 20 minutes	...	...	2	...	3	1
2	D	Rabbit ...	Unheated	4	3	...	5	...	2
			60° C. for 20 minutes	...	2	...	2	...	3
3	A	Sheep... ...	Unheated	3	...	...	3	...	2
			56° C. for 20 minutes	...	...	...	5	...	3
4	D	Monkey ...	Unheated	3	...	...	2	...	2
			64° C. for 20 minutes	...	...	...	3	...	3
5	A	Fowl	Unheated	6	...	7	5	...	3
			56° C. for 20 minutes	...	...	4	5	...	2
6	A	*Varanus* ...	Unheated	12	2	...	4	6	...
		Rabbit	Unheated	...	13	...	17	15	...
7	D	Baboon ...	Unheated	6	...	...	3	...	0*
			48° C. for 20 minutes	...	...	...	3	...	2
			56° C. for 20 minutes	...	...	...	4	..	4

* Signifies an unusually heavy bacterial growth.

forth the results of some experiments on the gut and on the gland forms respectively of this strain. Study of these two Tables reveals the fact that the behaviour of the developmental forms of *T. gambiense* in *G. palpalis* is similar to that of *T. rhodesiense,* that is that the gut

TABLE VI

Showing the effect of sera deactivated in different ways on the gut forms of a strain of *T. gambiense* from *G. palpalis*, *in vitro* at room temperature

Fly	Serum	Treatment of serum	Number of living trypanosomes per 256 squares of the haemocytometer scale					
			Start	1 hour	2 hours	4 hours	6 hours	24 hours
1	Rabbit ...	Untreated	30	0	...	...	...	...
		56° C. for 20 minutes	...	16	28	30	...	35
		64° C. for 20 minutes	...	18	22	19	...	18
2	Rabbit ...	Untreated	26	0	...	...	...	...
		Filtered	...	30	...	42	...	22
		62° C. for 20 minutes	...	...	...	...	...	26
3	Rabbit ...	Untreated	27	0	...	...	...	...
		NH_4OH	...	26	...	4	...	0
		56° C for 20 minutes	...	28	...	26	...	28
4	Sheep ...	Untreated	12	0	...	...	...	...
		NH_4OH	...	15	...	17	...	0*
		56° C. for 20 minutes	...	17	...	18	...	20*
5	Monkey ...	Untreated	26	0	...	...	...	...
		56° C. for 20 minutes	...	20	...	28	30	26
		64° C. for 20 minutes	...	22	...	24	24	16
6	Goat	Untreated	22	0	...	0	...	...
		Filtered	...	7	...	6	5	0*
		56° C. for 20 minutes	...	28	...	15	16	8*
		64° C. for 20 minutes	...	18	...	16	18	11
7	Fowl	Untreated	3	0	...	...	...	...
		Filtered	...	1	2	4	2	2
		56° C. for 20 minutes	...	5	4	...	1	1
		64° C. for 20 minutes	...	4	8	...	2	5
8	Crocodile ...	Untreated	8	0	...	...	...	...
		Filtered	...	7	...	8	...	0*
		64° C. for 20 minutes	...	28	...	18	...	28
9	Baboon ...	Untreated	56	1	0	...	...	0
		64° C. for 20 minutes	...	48	...	...	64	48
10	Human ...	Untreated	20	0	...	...	...	...
		56° C. for 10 minutes	...	12	...	...	12	0*
		64° C. for 20 minutes	...	14	...	...	18	34

* Signifies an unusually heavy bacterial growth.

forms survive for a period of at least twenty-four hours in deactivated sera, but are killed in under one hour by any of the fresh sera used, while the gland forms are completely resistant to fresh sera, over the period of observation.

TABLE VII.

Showing the effect of various sera on salivary gland forms of a strain of *T. gambiense* from *G. palpalis*, *in vitro*, at room temperature.

Fly	Serum	Treatment of Serum	Number of living trypanosomes per 256 squares of the haemocytometer scale					
			Start	1 hour	2 hours	4 hours	6 hours	24 hours
1	Rabbit ...	Unheated	13	15	...	18	...	18
		64° C. for 20 minutes	...	17	...	18	...	19
2	Sheep ...	Unheated	7	5	...	...	6	3
		56° C. for 20 minutes	...	6	...	...	4	2
3	Monkey ...	Unheated	3	2	...	4	...	2
		64° C. for 20 minutes	...	3	...	5	...	3
4	Goat	Unheated	24	24	...	18	20	16
		64° C. for 20 minutes	...	...	23	22	18	11
5	Fowl	Unheated	14	11	10	14	13	3
		64° C. for 20 minutes	...	12	12	10	9	5
6	Baboon ...	Unheated	4	...	3	...	2	0
		64° C. for 20 minutes	...	...	4	...	5	3

Experiments conducted by Duke, and as yet unpublished, show that once flies are infected with a trypanosome subsequent feeds of blood on animals, even themselves naturally immune to the parasite, fail to dislodge the infection. In view of the foregoing experiments it is remarkable that any gut infection, with the trypanosomes here considered, manages to survive the variety of feeds of blood that must be taken up by the infected fly. It would appear that ingested blood must be so altered that it is deprived of its trypanolytic properties by the time it reaches the infected portion

of the gut. In no other way can the failure of 'clean' feeds to sterilise the fly be explained. In order to determine whether this alteration in the nature of the ingested blood does indeed occur, some batches of clean flies were given full feeds of blood on a clean guinea-pig, the batches were then killed at varying periods after feeding and rapidly dissected. The ingested blood from the crops and mid-guts of a number of flies was removed into a small quantity of normal saline and examined by Dr. A. Wormall for the presence of haemolytic complement. It was found that within ten minutes of feeding the complement activity of the ingested material had disappeared, while control samples of blood from the guinea-pig, diluted to a similar extent with normal saline, exhibited the presence of a normal amount of complement. It would thus appear that complement is very rapidly altered after ingestion.

A number of recently hatched unfed flies were next dissected and the salivary glands and mid-guts of about thirty were pooled in 0·2 c.c. and in 0·5 c.c. of normal saline respectively and thoroughly emulsified. After centrifuging each emulsion the supernatant fluid was removed and examined by Dr. Wormall, who reports that there was present an anti-complementary substance in the mid-gut extract to a marked degree, and in the salivary gland extract to a lesser degree. The presence of this substance would therefore account for the failure to detect complement in the samples of ingested blood. Further work is being undertaken to elucidate the nature of this anti-complementary body and will be published in due course, but at present it seems clear that there is present in the salivary glands and mid-intestines of *Glossina palpalis* an anti-complementary substance which rapidly alters the complement of freshly ingested blood and so renders the blood non-trypanolytic to any gut forms of *T. rhodesiense* and *T. gambiense* which may be situated in the fly's intestine.

SUMMARY

1. A technique is described whereby the action of various sera on the gut and salivary gland forms of the pathogenic trypanosomes from *G. palpalis* can be studied *in vitro*.

2. It is shown that these forms, in a suitable medium, can be maintained alive *in vitro* for a period of at least twenty-four hours at

laboratory temperatures (24° C. to 29° C.). They usually survive in spite of the considerable bacterial invasion often unavoidably present, and in fairly sterile media generally show definite evidence of multiplication.

3. The fresh sera of a number of different mammals, birds, and reptiles are rapidly trypanocidal to the gut forms of *T. rhodesiense* and *T. gambiense* recovered from laboratory infected *G. palpalis*.

4. Heating these sera to 56° C. for twenty minutes, filtering them through a Berkefeld filter candle, or treating them with ammonia destroys their trypanocidal powers. As haemolytic complement is removed by these procedures, but was found to be present in the trypanocidally active sera, it is concluded that complement is the trypanocidal body.

5. These same sera, even when undeactivated, exert no lytic action on the salivary gland forms of *T. rhodesiense* and *T. gambiense in vitro*. The gland forms are thus shown to differ fundamentally from the gut forms; were this not the case, it would be difficult to account for the infection of mammals by the injection of salivary gland forms.

6. It has been shown that blood, recovered from recently fed *G. palpalis*, is devoid of haemolytic complement, no complement being detected in blood obtained from flies within ten minutes of feeding.

7. The presence of an anti-complementary substance has been demonstrated in saline emulsions of the mid-intestines, and to a less extent in those of the salivary glands, of unfed *G. palpalis*, and it is probably due to the presence of this substance that infected flies are not sterilised by subsequent feeds of normal complement-containing blood.

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STUDIES IN CHEMOTHERAPY*

IV.—THE ACTION *IN VIVO* OF CERTAIN ARSENICAL AND ANTIMONIAL COMPOUNDS AND OF BAYER 205 ON *T. RHODESIENSE* AND ON ATOXYL- AND ACRIFLAVINE-RESISTANT STRAINS OF THIS PARASITE

BY

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(*Received for publication* 25 *May*, 1931)

In a recent paper (1930) we recorded the trypanocidal action *in vitro* of a number of preparations of arsenic and antimony on *T. rhodesiense*, and on atoxyl- and acriflavine-resistant strains of this parasite. As the result of this work certain conclusions were reached of which the following are probably the most important :—

1. The organic pentavalent compounds are *per se* but slightly trypanocidal, whilst the corresponding trivalent compounds are powerfully trypanocidal.

2. The response of each of the two resistant strains to the various compounds, although frequently very different from that of the normal strain, proved in every case to be exactly similar to one another. The resistant strains were but slightly more resistant to the pentavalent arsenicals and to aniline than was the normal strain, withstanding as a rule about twice the concentration of drug as did the latter strain. But to the various trivalent arsenicals and arsenobenzols the resistant strains exhibited extraordinarily different degrees of resistance, e.g., to reduced atoxyl thioglycollate, *p*-aminophenylarsenoxide and arsenophenylglycine, they were only about two to eight times as resistant as was the normal strain, whereas to arsenophenylglycineamide and to reduced tryparsamide thioglycollate they were no less than 256 to 1,024 times as resistant as was the normal strain. To sodium arsinite and to tartar-emetic the resistant strains proved to be just as susceptible as did the normal strain.

3. The term '*arsenic resistance*' as applied to atoxyl-resistant and acriflavine-resistant strains is a definite misnomer. These strains are not arsenic resistant, but resistant to various organic compounds of arsenic. The similarity of the behaviour of the two strains made resistant to atoxyl and acriflavine respectively, the varying degree of resistance which they exhibit to the different organic compounds of arsenic,

* This work was assisted by a grant from the Chemotherapy Committee of the Medical Research Council.

and the fact that they are just as susceptible to sodium arsinite as is the normal strain, all indicate in the clearest possible manner that the resistance of these strains is not to arsenic, but to the substituted phenyl radical.

In the present work, using the same strains* and the same drugs (with certain additional compounds)†, we have endeavoured, so far as possible, to re-examine the validity of these deductions by experiments *in vivo*.

The animal used was the mouse, and in every case a single dose of the drug was given intraperitoneally in 1 c.c. of water. So far as possible the mice were treated when the blood showed a moderate infection of one to twenty parasites per microscope field (1/6 obj. and 4 oc.). It will, however, be obvious that in work which necessitated the use of enormous numbers of animals, it was not always found practicable to keep within these strict limits. The doses given in the tables represent in milligrams the dose per 20 gram mouse. The blood of the mice was examined at frequent intervals during the first twenty-four hours, and subsequently at least once daily during the remainder of life, or up to a period of thirty days after treatment when, if there was no relapse, the animals were regarded as cured.

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* *T. rhodesiense :* normal, atoxyl- and acriflavine-resistant strains. *Anns. Trop. Med. & Parasitol.*, **24**, 455.

† The drugs used were :—

Atoxyl = sodium 4-amino-phenyl-arsonate.
Reduced atoxyl thioglycollate = Disodium di (carboxymethyl) 4-amino-phenyl-thioarsinite.
Sodium 4-hydroxy-phenyl-arsonate.
4-hydroxy-phenyl-arsenoxide.
Arsacetin = sodium 4-acetylamino-phenyl-arsonate.
Reduced arsacetin = 4-acetylamino-phenyl-arsenoxide.
Tryparsamide = sodium 4-glycineamido-phenyl-arsonate.
Reduced tryparsamide thioglycollate = Disodium di (carboxymethyl) 4-glycineamido-phenyl-thioarsinite.
Sodium stovarsol = sodium 3-acetylamino-4-hydroxy-phenyl-arsonate.
Reduced stovarsol sodium thioglycollate = Disodium di (carboxymethyl) 4-acetylamino-3-hydroxy-phenyl-thioarsinite.
Halarsol = 4-hydroxy-3-amino-phenyl-dichlorarsine hydrochloride.
Halarsol thioglycollate = Disodium di (carboxymethyl) 4-hydroxy-3-amino-phenyl-thioarsinite.
Novarsenobillon = sodium 3 : 3′ diamino-4 : 4′ dihydroxy-arsenobenzene methylene sulphoxylate.
Disodium 4-glycine-phenyl-arsonate.
Arsenophenylglycine = sodium 4 : 4′-glycine-arsenobenzene.
Sodium arsinite.
Stibenyl = sodium 4-acetylamino-phenyl-stibinate.
Stibosan = sodium 4-acetylamino-3-chlor-phenyl-stibinate.
Tartar emetic = antimony potassium tartrate.
Acriflavine = 2 : 8 diamino methyl-acridinium chloride mono-hydrochloride.
Bayer 205.

We are greatly indebted to Dr. Ewins, of Messrs. May & Baker, for kindly preparing many of these compounds, and for much assistance and advice.

It will at once be recognised that this type of work is subject to various limitations not encountered with *in vitro* experiments. In the first place, in the latter class of experiment there is no limit, apart from that dependent upon solubility, to the concentration of the drug which can be used. The importance of this fact is immediately appreciated when we have to deal with a drug like reduced tryparsamide thioglycollate, to which the resistant strains proved *in vitro* to be no less than 256 to 1,000 times as resistant as did the normal strain. In experiments *in vivo* there is a very definite limit to the concentration of drug which can be employed, this limit being due to the toxic action of the drug on the host. Unfortunately, in the case of certain drugs, this limitation has prevented our ascertaining even the dose of certain drugs required to sterilize the peripheral blood of mice infected with the resistant strains.

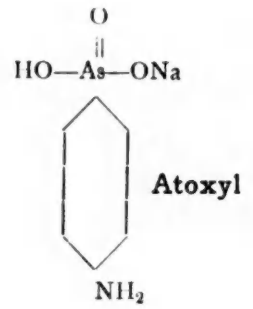
In the second place, the intraperitoneal introduction into mice of 1 c.c. of water containing a certain quantity of a drug provides no information regarding the actual concentration or the drug reached in the circulating blood of the mouse, or how long that concentration is maintained. These questions obviously largely depend on the rate of excretion of the drugs, and there is reason to believe that it differs widely, not only in the case of different compounds, but also in the case of individual mice. We have, unfortunately, very little knowledge on these important questions, but the work of Voegtlin (1925), Fordyce, Rosen and Myers (1924), Colebrook and Roche Lynch (1928) and others, possibly throw some light on the matter.

Before passing to a presentation and consideration of our protocols, it is necessary to define certain terms in the *special and restricted* sense in which each is used.

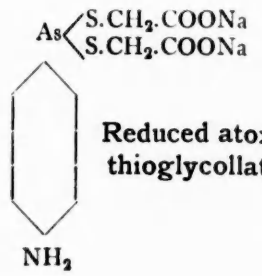
Minimum Effective Dose (M.E.D.). The minimum dose which sufficed to clear the blood of at least 80 per cent. of the treated mice, irrespective of time.

Minimum Curative Dose (M.C.D.). The minimum dose which sufficed to cure (thirty days' observation) at least 80 per cent. of the treated mice.

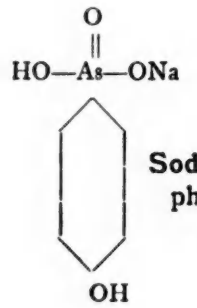
Lethal Dose (L.D.). The minimum dose which killed at least 50 per cent. of the treated mice within twenty-four hours in the case of the inorganic, trivalent organic, and arsenobenzol compounds,



Atoxyl

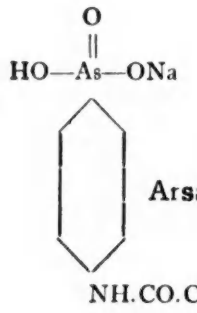


Reduced atoxyl thioglycollate



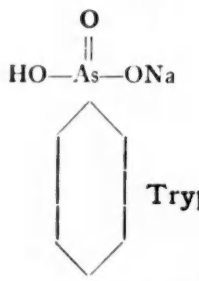
Sodium 4-hydroxyphenylarsonate

4-hydroxyphenylarsenoxide

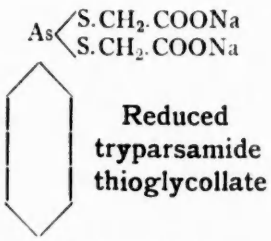


Arsacetin

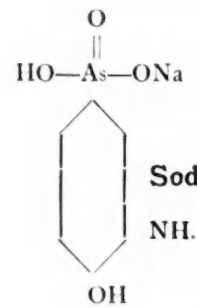
Reduced arsacetin



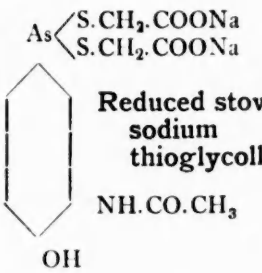
Tryparsamide



Reduced tryparsamide thioglycollate

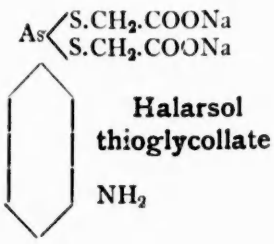


Sodium stovarsol

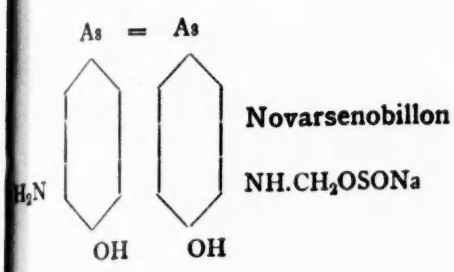


Reduced stovarsol sodium thioglycollate

Halarsol

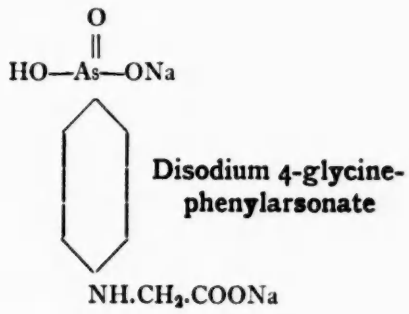


Halarsol thioglycollate

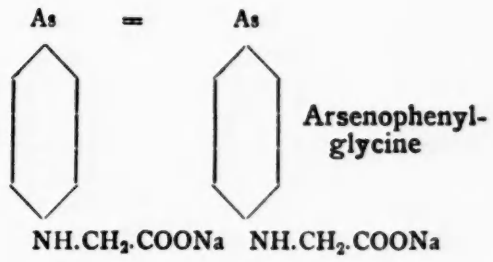


Novarsenobillon

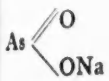
$NH.CH_2OSONa$



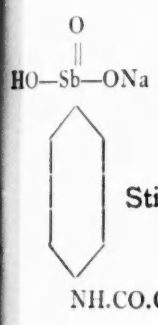
Disodium 4-glycine-phenylarsonate



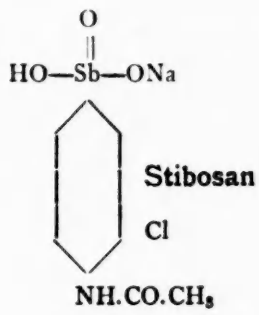
Arsenophenyl-glycine



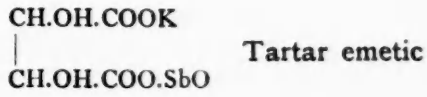
Sodium arsinite



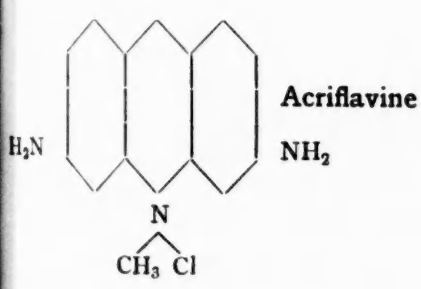
Stibenyl



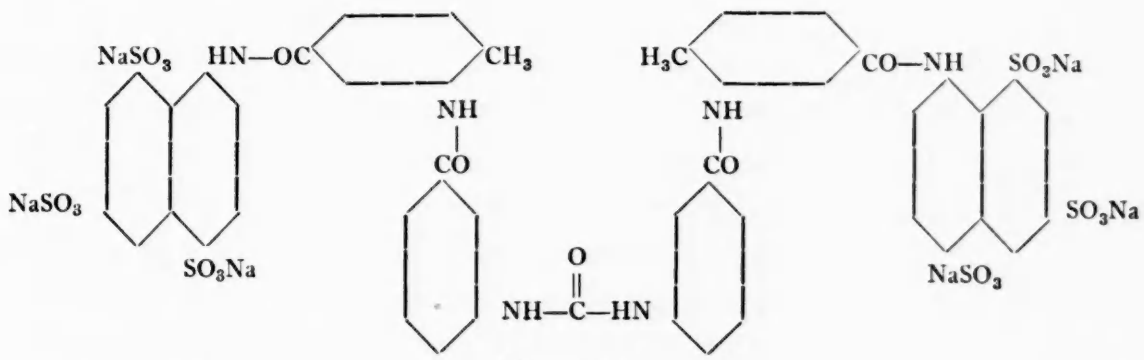
Stibosan



Tartar emetic



Acriflavine



Bayer 205

and within three to four days in the case of the pentavalent organic compounds.

It must be noted that we are in this paper using the expression 'Lethal dose' in an entirely special sense. The determination of this was necessary in order that we could decide the dose beyond which it was useless to go in our endeavour to ascertain the relative resistance of the atoxyl- and acriflavine-resistant strains, as compared with that of the normal strain. In arriving at the figures for the lethal doses given in the tables, the question of time is obviously one of great importance. As stated in the definition we decided, in determining the lethal dose of the inorganic, trivalent organic, and arsenobenzol compounds, all of which *in vitro* are powerfully trypanocidal—very weak solutions sufficing to destroy the trypanosomes within a few hours—to limit the observation to a period of twenty-four hours. This period appeared to us to be adequate; firstly, because the trypanocidal action of these compounds is so rapid that if they have been administered in sufficient amount to produce any definite effect, that effect will be manifest in considerably less than twenty-four hours, and, secondly, because experience has shown that these active drugs kill mice quickly if they are administered in lethal doses; as a rule, if the animals survive twenty-four to forty-eight hours they survive altogether. In the case of the slow-acting pentavalent compounds which require to be changed—probably into their corresponding reduced products—before they become trypanocidal, it is quite obvious that for the purpose we have in view the limiting of the observation to a period of twenty-four hours would be useless. Many of these drugs, especially when given in doses which are bordering on the minimum effective dose, require several days to produce sterilization of the peripheral circulation. Furthermore, the pentavalent compounds differ from the active trivalent compounds and from the arsenobenzols in that when given in lethal doses—provided the dose is not exceedingly large—they do not kill, as do the trivalent compounds, within twenty-four hours, but only after periods varying from two or three to six or seven days. The truth of this is shown by the results obtained from the injection of 138 normal mice with doses of atoxyl varying from 10 to 20 mgm. per 20 gm. of body weight. Of these, fifty-two (37·5 per cent.) died; one animal died on the first day after

injection, nine on the second day, nineteen on the third day, seven on the fourth day, eight on the fifth day, six on the sixth day, and two on the seventh day.

The results of the work with which this paper deals are summarized in the following tables which require but little explanation. The statement ' infection controlled ' sometimes occurring in the remarks column, implies that although the blood of the treated mice failed to become negative, nevertheless, the dose of drug given exerted some influence on the infection in that the trypanosomes failed to increase in numbers at the rate usual in untreated mice. ' Dose lethal ' implies that at least 50 per cent. of the treated mice died—presumably from the poisonous effects of the drug—within the various periods mentioned in the definition of the expression as employed by us.

In Table XXII we have given the M.E.D. and M.C.D. of the various drugs for infections due to the normal strain, and to those made resistant to atoxyl and acriflavine respectively. It should at once be noted that the last two proved to be identical, so far as their reaction to the various drugs employed is concerned. We have also recorded in this table the concentrations of the drugs necessary to kill *in vitro*, at 37° C. within a period of twenty-four hours, the normal and resistant trypanosomes. In the last columns we have calculated the ' resistant factors ' obtained from the *in vivo* and *in vitro* experiments respectively ; the ' resistant factors ' in the former are obtained from the fraction $\frac{\text{M.E.D. for the resistant strains}}{\text{M.E.D. for the normal strain}}$ and those for the latter from the fraction

$$\frac{\text{Lethal conc. of drug for the resistant strains}}{\text{Lethal conc. of drug for the normal strain.}}$$

Study of those portions of the table relating to the *in vivo* experiments shows at once that, for the purpose we had in view, viz., the comparison of the degree of resistance to various drugs exhibited by the different strains *in vivo* and *in vitro*, the *in vivo* work suffered from pronounced limitations determined by the lethal doses of the drugs. In the case of the normal strain, it was found possible to obtain the M.E.D. of all but one of the drugs used, viz., sodium arsinite, and the M.C.D. of all but five, viz., sodium arsinite, reduced atoxyl thioglycollate, sodium stovarsol, disodium 4-glycine-phenyl-

TABLE I

ATOXYL in the treatment of mice infected with *T. rhodesiense* (normal, atoxyl- and acriflavine-resistant strains.

Strain.	Dose per 20 gm. mouse. mgm.	Degree of infection of mouse when treated. Parasites per field. (1/6 obj. 4 oc.)	Number of mice treated.	Number of mice which became negative.	Time taken to become negative. Hours.	Length of time mice remained negative. Days.	Remarks.
Normal strain.	1·0	10 to ++	5	0			
	2·0	1 to ++	10	8	6-72	3 mice : 2 1 mouse : 5 2 mice : 8 2 mice : cured	
	3·0	8 to +	5	5	7-72	1 mouse : 3 1 mouse : 5 1 mouse : 10 2 mice : cured	
	4·0	1 to 20	10	10	6	2 mice : 4† 1 mouse : 8† 2 mice : 10 5 mice : cured	
	6·0	1/5 to +	5	5	6-24	1 mouse : 8 1 mouse : 12† 3 mice : cured	
Atoxyl-resistant strain.	10·0	1 to 10	5	0			
	15·0	1/10 to 20	5	0	...	...	Dose lethal : infection in survivors controlled
	20·0	1/3 to 20	10	0	...	...	Dose lethal ; infection in survivors controlled
Acriflavine-resistant strain.	10·0	1/10 to 10	5	1	48-72	1 mouse : 2†	
	15·0	2 to 5	5	0	...	...	Dose lethal ; infection in survivors controlled
	20·0	1/10 to 10	5	1	48-72	1 mouse : 1	Dose lethal ; infection in survivors controlled

† Mouse died without relapse

Table I shows that for atoxyl—
Lethal dose = 10 to 15 mgm.
For infections due to the normal strain—
M.E.D. = 2 mgm.
M.C.D. = 6 mgm.
For infections due to the resistant strains—
M.E.D. = 10 + mgm.

∴ Resistant factor = 5+

TABLE II

REDUCED ATOXYL THIOGLYCOLLATE in the treatment of mice infected with *T. rhodesiense* (normal, atoxyl- and acriflavine-resistant strains.

Strain.	Dose per 20 gm. mouse. mgm.	Degree of infection of mouse when treated. Parasites per field. (1/6 obj. 4 oc.)	Number of mice treated.	Number of mice which became negative.	Time taken to become negative. Hours.	Length of time mice remained negative. Days.	Remarks.
Normal strain.	0·05	1/5 to 20	5	0	...	...	Infection markedly controlled.
	0·075	20 to ++	5	5	12-36	2 mice : 3 2 mice : 6 1 mouse : 9	
	0·1	2 to +	5	5	12-36	3 mice : 5 2 mice : 7	
	0·2	5 to 20	5	5	2-4	...	Dose lethal; all mice dead in 6 to 12 hours
Atoxyl-resistant strain.	0·1	1 to 15	5	0			
	0·15	10 to 20	5	0	...	...	Dose lethal; all mice dead in 6 to 24 hours.
	0·2	8 to 15	5	0	...	...	Dose lethal; all mice dead in 6 to 24 hours.
Acriflavine-resistant strain.	0·1	1/5 to 5	5	0			
	0·15	1/5 to 30	5	0	...	...	Dose lethal; all mice dead in 6 to 24 hours.

Table II shows that for reduced atoxyl thioglycollate—

Lethal dose = 0·15 mgm.

For infections due to the normal strain—

M.E.D. = 0·075 mgm.

M.C.D. not obtainable.

For infections due to the resistant strains—

M.E.D. = 0·15 ++ mgm.

∴ Resistant factor = 2 ++

TABLE III

SODIUM 4-HYDROXY-PHENYL-ARSONATE in the treatment of mice infected with *T. rhodesiense* (normal, atoxyl- and acriflavine-resistant strains)

Strain.	Dose per 20 gm. mouse. mgm.	Degree of infection of mouse when treated. Parasites per field. (1/6 obj. 4 oc.)	Number of mice treated.	Number of mice which became negative.	Time taken to become negative. Hours.	Length of time mice remained negative. Days.	Remarks.
Normal strain	1·0	1/2 to +	7	4	12-48	1 mouse : 4 1 mouse : 5 1 mouse : 6 1 mouse : 15	
	2·0	20 to ++	2	2	3-6	1 mouse : 8 1 mouse : 19	
	4·0	1 to +	4	4	3-6	1 mouse : 7 1 mouse : 17 1 mouse : 29 1 mouse : cured	
	5·0	1/20 to 1/2	5	5	2-4	1 mouse : 21 4 mice : cured	
	6·0	1/7 to ++	5	5	2-4	2 mice : 3† 3 mice : cured	
	7·5	2 to 5	5	5	2-4	5 mice : cured	
	10·0	1/5 to ++	6	6	2-4	3 mice : 1† 3 mice : cured	
	15·0	1/20 to 10	5	5	2-4	2 mice : 1† 3 mice : 2†	Dose lethal.
Atoxyl-resistant strain	6·0	1/30 to 5	3	0	...	...	Infection uninfluenced.
	10·0	1/30 to 5	3	0	...	...	Infection uninfluenced.
Acriflavine-resistant strain	10·0	5 to 15	5	0	...	...	Infection uninfluenced.

† Mouse died without relapse.

Table III shows that for sodium 4-hydroxy-phenyl-arsonate—

Lethal dose = 10 to 15 mgm.

For infections due to the normal strain—

M.E.D. = 1·5 mgm.

M.C.D. = 5 mgm.

For infections due to the resistant strains—

M.E.D. = 10 ++ mgm.

∴ Resistant factor = 7 ++

TABLE IV

4-HYDROXY-PHENYL-ARSENOXIDE in the treatment of mice infected with *T. rhodesiense* (normal, atoxyl- and acriflavine-resistant strains)

Strain.	Dose per 20 gm. mouse. mgm.	Degree of infection of mouse when treated. Parasites per field. (1/6 obj. 4 oc.)	Number of mice treated.	Number of mice which became negative.	Time taken to become negative. Hours.	Length of time mice remained negative. Days.	Remarks.
Normal strain.	0·025	2 to +	5	3	36-60	2 mice : 2 1 mouse : 3	
	0·0375	1 to 5	5	5	4-12	1 mouse : 2 3 mice : 5 1 mouse : 11	
	0·05	1/2 to 10	5	5	2-8	4 mice : 10 1 mouse : cured	
	0·1	1 to 10	5	5	1-4	1 mouse : 11 1 mouse : 13 3 mice : cured	
	0·15	5 to +	5	5	1-2	1 mouse : 5† 1 mouse : 9†	3 mice dead in 3 to 48 hours.
	0·2	1 to 20	4	4	1-2	...	Dose lethal : all mice dead in 24 hours.
	0·4	2 to 20	4	4	1-2	...	Dose lethal : all mice dead in 2 to 4 hours.
Atoxyl-resistant strain.	0·15	1 to +	5	0	...	...	Infection uninfluenced.
Acriflavine-resistant strain.	0·15	2 to ++	5	0	...	...	Infection uninfluenced.

† Mouse died without relapse.

Table IV shows that for 4-hydroxy-phenyl-arsenoxide—

Lethal dose = 0·2 mgm.

For infections due to the normal strain—

M.E.D. = 0·0375 mgm.

M.C.D. = 0·1+ mgm.

For infections due to the resistant strains—

M.E.D. = 0·15++ mgm.

∴ Resistant factor = 4++

TABLE V

ARSACETIN in the treatment of mice infected with *T. rhodesiense* (normal, atoxyl- and acriflavine-resistant strains)

Strain.	Dose per 20 gm. mouse. mgm.	Degree of infection of mouse when treated. Parasites per field. (1/6 obj. 4 oc.)	Number of mice treated.	Number of mice which became negative.	Time taken to become negative. Hours.	Length of time mice remained negative. Days.	Remarks.
Normal strain	1·5	3 to ++	5	0	...	...	
	3·0	5 to ++	10	5	36-48	1 mouse : 2 1 mouse : 3 2 mice : 4 1 mouse : cured	
	4·0	1 to +	10	9	4-72	1 mouse : 2 1 mouse : 4 1 mouse : 5 6 mice : cured	
	5·0	1 to +	5	5	3-6	5 mice : cured	
Atoxyl-resistant strain	30·0	+ to ++	3	0	...	...	
	40·0	1/20 to 10	5	0	...	...	Infection slightly controlled.
	50·0	1/20 to 15	7	1	36-48	1 mouse : 5	Dose lethal; 6 mice dead in 6 to 24 hours.
	75·0	1/10 to +	8	0	...	...	Dose lethal; 6 mice dead in 3 to 12 hours.
Acriflavine-resistant strain	50·0	1/5 to 2	5	0	...	...	Dose lethal; 2 mice dead in 6 to 24 hours.

Table V shows that for arsacetin—

Lethal dose = 50 mgm.

For infections due to the normal strain—

M.E.D. = 4 mgm.

M.C.D. = 5 mgm.

For infections due to the resistant strains—

M.E.D. = 40 + mgm.

∴ Resistant factor = 10 +

TABLE VI

REDUCED ARSACETIN in the treatment of mice infected with *T. rhodesiense* (normal, atoxyl- and acriflavine-resistant strains)

Strain.	Dose per 20 gm. mouse. mgm.	Degree of infection of mouse when treated. Parasites per field. (1/6 obj. 4 oc.)	Number of mice treated.	Number of mice which became negative.	Time taken to become negative. Hours.	Length of time mice remained negative. Days.	Remarks.
Normal strain	0·0125	1/5 to 4	5	0	...	...	Infection controlled.
	0·025	1/20 to 10	5	4	4-72	3 mice : 6 1 mouse : 7	
	0·05	1/3 to 10	7	7	1-6	3 mice : 9 1 mouse : 13† 1 mouse : 13 1 mouse : 20 1 mouse : cured	
	0·1	1/10 to 10	7	7	1-3	2 mice : 10† 1 mouse : 12 4 mice : cured	
	0·5	3 to ++	7	7	1-3	...	Dose lethal; all mice dead in 3 to 6 hours.
Atoxyl-resistant strain	0·375	1/3 to ++	5	0	...	...	Dose lethal; all mice dead in 6 to 24 hours.
Acriflavine-resistant strain	0·25	10 to 15	3	0	...	...	Infection uninfluenced.
	0·375	1/10 to 5	5	0	...	...	Dose lethal; all mice dead in 12 to 24 hours.
	0·5	1/3 to 10	6	0	...	...	Dose lethal; all mice dead in 6 to 12 hours.

† Mouse died without relapse.

Table VI shows that for reduced arsacetin—
Lethal dose = 0·375 mgm.
For infections due to the normal strain—
M.E.D. = 0·025 mgm.
M.C.D. = 0·1 mgm.
For infections due to the resistant strains—
M.E.D. = 0·375++

∴ Resistant factor = 15++

TABLE VII

TRYPARSAMIDE in the treatment of mice infected with *T. rhodesiense* (normal, atoxyl- and acriflavine-resistant strains)

Strain.	Dose per 20 gm. mouse. mgm.	Degree of infection of mouse when treated. Parasites per field. (1/6 obj. 4 oc.)	Number of mice treated.	Number of mice which became negative.	Time taken to become negative. Hours.	Length of time mice remained negative. Days.	Remarks.
Normal strain	5·0	1/2 to +	5	2	7-72	1 mouse : 2 1 mouse : 16†	Infection slightly controlled.
	6·0	2 to 20	5	5	6-24	2 mice : 6 2 mice : 7 1 mouse : 9	
	10·0	5 to ++	5	5	6-12	2 mice : 8† 2 mice : 9 1 mouse : cured	
	12·5	2 to 10	5	5	3-12	3 mice : 7 2 mice : cured	
	15·0	2 to 15	5	5	5-12	1 mouse : 2 1 mouse : 7 1 mouse : 10† 2 mice : cured	
	20·0	1/5 to 8	5	5	4-6	1 mouse : 12† 4 mice : cured	
Atoxyl-resistant strain	50·0	+	2	0			
	75·0	+	2	0			
	100·0	1/20 to +	9	4	6-48	3 mice : 1† 1 mouse : 8	Dose lethal : infection in survivors controlled.
	125·0	1/10 to 20	8	4	12-24	4 mice : 1†	Dose lethal ; infection in survivors controlled.
	150·0	1 to 2	3	1	12-24	1 mouse : 1†	Dose lethal ; infection in survivors controlled.
Acriflavine-resistant strain	100·0	3 to 10	5	0	...	...	Dose lethal ; infection in survivors controlled.
	125·0	1/20 to 2	5	1	6-12	1 mouse : 1†	Dose lethal ; infection in survivors controlled.

† Mouse died without relapse.

Table VII shows that for tryparsamide—
Lethal dose = 100 mgm.
For infections due to the normal strain—
M.E.D. = 6 mgm.
M.C.D. = 20 mgm.
For infections due to the resistant strains—
M.E.D. = 100+ mgm.

∴ Resistant factor = 16+

TABLE VIII

REDUCED TRYPARSAMIDE THIOGLYCOLLATE in the treatment of mice infected with *T. rhodesiense* (normal, atoxyl- and acriflavine-resistant strains)

Strain.	Dose per 20 gm. mouse. mgm.	Degree of infection of mouse when treated. Parasites per field. (1/6 obj. 4 oc.)	Number of mice treated.	Number of mice which became negative.	Time taken to become negative. Hours.	Length of time mice remained negative. Days.	Remarks.
Normal strain	0·025	10 to +	5	0			
	0·03	1/5 to ++	5	3	12-48	2 mice : 1 1 mouse : 2	Infection controlled.
	0·04	1 to 10	5	5	2-4	2 mice : 2 3 mice : 5	
	0·06	1 to 15	5	5	2-4	1 mouse : 3 3 mice : 5 1 mouse : 7	
	0·1	1/5 to 10	5	5	1-3	2 mice : 6 3 mice : 9	
	0·25	1/4 to 5	5	5	1-3	2 mice : 7 1 mouse : 9 2 mice : cured	
	0·5	5 to 10	5	5	1-3	1 mouse : 12† 4 mice : cured	
Atoxyl-resistant strain	0·8	8 to ++	4	0			
	2·0	1 to 20	8	0	...	...	Infection uninfluenced.
	3·0	3 to +	5	0	...	...	Dose lethal, all mice dead in 6-24 hours.
	4·0	5 to 8	3	0	...	...	Dose lethal, all mice dead in 6-24 hours.
Acriflavine-resistant strain	2·0	1/20 to 5	5	0	...	...	Infection uninfluenced.

† Mouse died without relapse.

Table VIII shows that for reduced tryparsamide thioglycollate—

Lethal dose = 3 mgm.

For infections due to the normal strain—

M.E.D. = 0·03 to 0·04 mgm.

M.C.D. = 0·5 mgm.

For infections due to the resistant strains—

M.E.D. = 2++ mgm.

∴ Resistant factor = 60++

TABLE IX

SODIUM STOVARSOL in the treatment of mice infected with *T. rhodesiense* (normal, atoxyl- and acriflavine-resistant strains)

Strain	Dose per 20 gm. mouse. mgm.	Degree of infection of mouse when treated. Parasites per field (1/6 obj. 4 oc.)	Number of mice treated.	Number of mice which became negative.	Time taken to become negative. Hours.	Length of time mice remained negative. Days.	Remarks.
Normal strain	5·0	2 to 10	5	0			
	10·0	8 to 20	5	1	8-9	1 mouse : 4	
	15·0	10 to +	5	3	6-9	1 mouse : 3 1 mouse : 4 1 mouse : 8	
	20·0	1 to +	10	5	6-24	1 mouse : 1† 2 mice : 2 1 mouse : 4 1 mouse : 7	
	30·0	4 to 15	5	5	4-12	1 mouse : 1† 1 mouse : 2† 1 mouse : 7 1 mouse : 9 1 mouse : cured	
	40·0	1 to 10	5	5	2-6	1 mouse : 1† 2 mice : 10 2 mouse : cured	
Atoxyl-resistant strain	50·0	1 to 10	5	0	...	...	Infection controlled.
	75·0	1/6 to ++	5	1	6-12	1 mouse : 1†	Dose lethal, 4 mice dead in 6-24 hours.
Acriflavine-resistant strain	75·0	1/2 to +	5	1	6-12	1 mouse : 1†	Dose lethal, 3 mice dead in 6-24 hours.

† Mouse died without relapse.

Table IX shows that for sodium stovarsol—
Lethal dose = 50 to 75 mgm.
For infections due to the normal strain—
M.E.D. = 30 mgm.
M.C.D. = not obtainable.
For infections due to the resistant strains—
M.E.D. = 50+ mgm.

∴ Resistant factor = 2+

TABLE X

REDUCED STOVARSOL THIOGLYCOLLATE in the treatment of mice infected with *T. rhodesiense* (normal, atoxyl- and acriflavine-resistant strains)

Strain.	Dose per 20 gm. mouse. mgm.	Degree of infection of mouse when treated. Parasites per field. (1/6 obj. 4 oc.)	Number of mice treated.	Number of mice which became negative.	Time taken to become negative. Hours.	Length of time mice remained negative. Days.	Remarks.
Normal strain	0·05	1 to +	10	0			
	0·075	1 to +	5	0			
	0·1	5 to 20	5	3	4-12	3 mice : 2	
	0·15	5 to 15	5	5	2-12	1 mouse : 1† 2 mice : 1 2 mice : 6	
	0·2	1/5 to +	5	5	1-3	2 mice : 4 3 mice : 6	
	0·4	1 to 10	5	5	1-3	1 mouse : 15 4 mice : cured	
Atoxyl-resistant strain	0·8	+ to ++	4	0	...	...	Infection slightly controlled.
	1·0	1 to 15	5	0	...	...	Dose lethal, 4 mice dead in 6-24 hours. In remaining mouse infection markedly controlled.
	1·5	1 to 15	5	1	3-6	1 mouse : 1†	Dose lethal, all mice dead in 12-24 hours.
	2·0	1/3 to 10	14	4	3-6	...	Dose lethal, all mice dead in 6 hours.
Acriflavine-resistant strain	1·0	1/10 to 5	5	1	3-6	...	Dose lethal, all mice dead in 12-24 hours.
	1·5	1/20 to 2	5	0	...	...	Dose lethal, all mice dead in 12-24 hours.
	2·0	3 to +	10	0	...	...	Dose lethal, all mice dead in 6 hours.

† Mouse died without relapse.

Table X shows that for reduced stovarsol thioglycollate—

Lethal dose = 1 mgm.

For infections due to the normal strain—

M.E.D. = 0·15 mgm.

M.C.D. = 0·4 mgm.

For infections due to the resistant strains—

M.E.D. = 0·8+ mgm.

∴ Resistant factor = 6+

TABLE XI

HALARSOL in the treatment of mice infected with *T. rhodesiense* (normal, atoxyl- and acriflavine-resistant strains)

Strain.	Dose per 20 gm. mouse. mgm.	Degree of infection of mouse when treated. Parasites per field. (1/6 obj. 4 oc.)	Number of mice treated.	Number of mice which became negative.	Time taken to become negative. Hours.	Length of time mice remained negative. Days.	Remarks.
Normal strain	0·0125	5 to 15	5	3	4-8	3 mice : 1	
	0·02	2 to ++	5	4	6-48	2 mice : 2 2 mice : 4	
	0·025	10 to +	5	5	3-4	3 mice : 4 2 mice : 7	
	0·05	2 to +	5	5	1-2	1 mouse : 5 3 mice : 9 1 mouse : 12	
	0·1	1/20 to +	5	5	1	5 mice : cured	
Atoxyl-resistant strain	0·75	1/5 to 1	5	0	...	...	Infection uninfluenced.
	1·0	1/5 to +	8	1	48	1 mouse : 1	Dose lethal, 3 mice dead in 6-24 hours.
	1·2	1 to ++	10	0	...	...	Dose lethal, 8 mice dead in 6-24 hours.
	1·5	10 to 20	3	0	...	...	Dose lethal, all mice dead in 6-12 hours.
Acriflavine-resistant strain	0·75	1/2 to 5	5	0	...	...	Infection uninfluenced.
	1·0	1/10 to 5	5	0	...	...	Dose lethal, all mice dead in 12 hours.

Table XI shows that for halarsol—

Lethal dose = 1 mgm.

For infections due to the normal strain—

M.E.D. = 0·02 mgm.

M.C.D. = 0·1 mgm.

For infections due to the resistant strains—

M.E.D. = 0·75++ mgm.

∴ Resistant factor = 37·5++

TABLE XII

HALARSOL THIOGLYCOLLATE in the treatment of mice infected with *T. rhodesiense* (normal, atoxyl- and acriflavine-resistant strains)

Strain.	Dose per 20 gm. mouse. mgm.	Degree of infection of mouse when treated. Parasites per field. (1/6 obj. 4 oc.)	Number of mice treated.	Number of mice which became negative.	Time taken to become negative. Hours.	Length of time mice remained negative. Days.	Remarks.
Normal strain	0·02	3 to +	5	2	24-48	2 mice : 1	
	0·03	1/20 to ++	4	3	3-36	2 mice : 3 1 mouse : cured	
	0·04	10 to 20	5	5	1-3	5 mice : 8	
	0·1	1/5 to 2	5	5	1-2	1 mouse : 8† 4 mice : cured	
Atoxyl-resistant strain	1·0	10 to +	4	0			
	1·2	5 to +	6	0	...	...	Infection uninfluenced.
	1·5	8 to 10	5	1	24-36	1 mouse : 1†	Dose lethal.
Acriflavine-resistant strain	1·5	1/5 to 10	5	2	24-36	2 mice : 1†	Dose lethal.
	2·0	3 to 10	5	1	6-12	1 mouse : 1†	Dose lethal, all mice dead in 24 hours.

† Mouse died without relapse.

Table XII shows that for halarsol thioglycollate—

Lethal dose = 1·5 mgm.

For infections due to the normal strain—

M.E.D. = 0·03 mgm.

M.C.D. = 0·1 mgm.

For infections due to the resistant strains—

M.E.D. = 1·2++ mgm.

∴ Resistant factor = 40++

TABLE XIII

NOVARSENOBILLON in the treatment of mice infected with *T. rhodesiense* (normal, atoxyl- and acriflavine-resistant strains)

Strain.	Dose per 20 gm. mouse. mgm.	Degree of infection of mouse when treated. Parasites per field. (1/6 obj. 4 oc.)	Number of mice treated.	Number of mice which became negative.	Time taken to become negative. Hours.	Length of time mice remained negative. Days.	Remarks.
Normal strain	0·06	2 to ++	5	2	72	2 mice : 2	
	0·08	5 to +	5	1	48	1 mouse : 1	
	0·1	10 to ++	5	3	24-72	3 mice : 2	
	0·125	2 to +	5	5	1-4	1 mouse : 7 1 mouse : 10 2 mice : 15 1 mouse : 18	
	0·25	10 to ++	4	4	2-5	1 mouse : 6 1 mouse : 9 2 mice : 18	
	0·5	10 to ++	4	4	2-5	1 mouse : 8 1 mouse : 13 2 mice : cured	
	1·0	5 to +	5	5	1-2	1 mouse : 1† 4 mice : cured	
Atoxyl-resistant strain	4·0	1 to +	5	1	72	1 mouse : 1	Infection controlled.
	5·0	1 to 10	11	4	20-48	1 mouse : 1 1 mouse : 2 1 mouse : 3 1 mouse : 4	Dose lethal, most of mice dead in 24 hours.
Acriflavine-resistant strain	4·0	1/3 to 3	5	0	...	...	Infection controlled.
	5·0	1/10 to 10	10	0	...	...	Dose lethal, most of mice dead in 24 hours.

† Mouse died without relapse.

Table XIII shows that for novarsenobillon—

Lethal dose = 5 mgm.

For infections due to the normal strain—

M.E.D. = 0·125 mgm.

M.C.D. = 1 mgm.

For infections due to the resistant strains—

M.E.D. = 4+ mgm.

∴ Resistant factor = 32+

TABLE XIV

DISODIUM 4-GLYCINE-PHENYL-ARSONATE in the treatment of mice infected with *T. rhodesiense* (normal, atoxyl- and acriflavine-resistant strains)

Strain.	Dose per 20 gm. mouse. mgm.	Degree of infection of mouse when treated. Parasites per field. (1/6 obj. 4 oc.)	Number of mice treated.	Number of mice which became negative.	Time taken to become negative. Hours.	Length of time mice remained negative. Days.	Remarks.
Normal strain.	20	1/30 to +	5	0			
	30	1/5 to +	5	0			
	40	1 to 20	5	2	4-6	1 mouse : 1† 1 mouse : cured	
	60	2 to +	5	5	4-6	1 mouse : 2 1 mouse : 5	Dose lethal : 3 mice dead in 24 hours.
Atoxyl-resistant strain.	20	1/2 to 20	5	0			
	40	5 to 15	5	2	4-6	1 mouse : 1 1 mouse : 2	
	60	2 to 10	5	4	4-6	2 mice : 1† 1 mouse : 4 1 mouse : 8	Dose lethal; 2 mice dead in 24 hours.
Acriflavine-resistant strain.	60	2 to 10	5	4	4-12	1 mouse : 2† 1 mouse : 3†	Dose lethal; 2 mice dead in 24 hours.

† Mouse died without relapse.

Table XIV shows that for disodium 4-glycine-phenyl-arsonate—

Lethal dose = 60 mgm.

For infections due to the normal srain—

M.E.D. = 60 mgm.

M.C.D. not obtainable.

For infections due to the resistant strains—

M.E.D. = 60 mgm.

∴ Resistant factor = 1

TABLE XV

ARSENOPHENYLGLYCINE in the treatment of mice infected with *T. rhodesiense* (normal, atoxyl- and acriflavine-resistant strains)

Strain.	Dose per 20 gm. mouse. mgm.	Degree of infection of mouse when treated. Parasites per field. (1/6 obj. 4 oc.)	Number of mice treated.	Number of mice which became negative.	Time taken to become negative. Hours.	Length of time mice remained negative. Days.	Remarks.
Normal strain	0·2	1/5 to 20	5	0			
	0·3	1/30 to 5	5	3	12-24	1 mouse : 2 1 mouse : 3 1 mouse : 5	
	0·4	8 to ++	9	4	6-12	3 mice : 1 1 mouse : 6	
	0·5	1/10 to +	15	13	2-4	4 mice : 5 6 mice : 7 1 mouse : 8 1 mouse: 11 1 mouse : cured	
	1·0	1 to +	5	5	1-2	1 mouse : 21 4 mice : cured	
	1·5	1/5 to 5	5	5	1-2	1 mouse : 4† 4 mice : cured	
Atoxyl-resistant strain	0·5	+ to ++	5	1	6-12	1 mouse : 1	
	0·6	1/30 to 15	5	5	1-3	3 mice : 2 1 mouse : 12 1 mouse : cured	
	0·8	1/5 to ++	14	10	1-6	7 mice : 2 2 mice : 7 1 mouse : 13	
	1·0	1/15 to 20	13	12	1-2	1 mouse : 2† 2 mice : 3 1 mouse : 5 1 mouse : 6† 5 mice : 10 1 mouse : 15 1 mouse : 21	
	1·5	2 to +	5	5	2-4	2 mice : 2† 1 mouse : 14 2 mice : cured	
	2·0	10 to +	5	5	3-4	...	Dose lethal.

TABLE XV—*continued*

ARSENOPHENYLGLYCINE in the treatment of mice infected with *T. rhodesiense* (normal, atoxyl- and acriflavine-resistant strains)

Strain	Dose per 20 gm. mouse. mgm.	Degree of infection of mouse when treated. Parasites per field (1/6 obj. 4 oc.)	Number of mice treated.	Number of mice which became negative.	Time taken to become negative. Hours.	Length of time mice remained negative. Days.	Remarks.
	0·3	1 to 5	5	2	6-12	1 mouse : 1 1 mouse : 4	
	0·5	1/20 to 15	9	7	2-6	5 mice : 2 2 mice : 5	
	0·6	1/20 to 3	5	5	1-3	2 mice : 5 3 mice : 9	
Acriflavine-resistant strain	0·8	1 to 15	5	5	1-3	1 mouse : 3 4 mice : 6	
	1·0	1/2 to 15	9	9	1-3	4 mice : 5 1 mouse : 5† 2 mice : 6 1 mouse : 9 1 mouse : 9†	
	1·5	1/15 to 5	5	5	1-3	1 mouse : 4† 1 mouse : 11 3 mice : cured	
	2·0	10 to +	5	5	2-4	4 mice : 1†	Dose lethal.

† Mouse died without relapse.

Table XV shows that for arsenophenylglycine—

Lethal dose = 2 mgm.

For infections due to the normal strain—

M.E.D. = 0·5 mgm.

M.C.D. = 1 mgm.

For infections due to the resistant strains—

M.E.D. = 0·5 to 0·6 mgm.

M.C.D. = 1·5+ mgm.

∴ Resistant factor = 1 to 2.

TABLE XVI

SODIUM ARSINITE in the treatment of mice with *T. rhodesiense* (normal, atoxyl- and acriflavine-resistant strains)

Strain.	Dose per 20 gm. mouse. mgm.	Degree of infection of mouse when treated. Parasites per field. (1/6 obj. 4 oc.)	Number of mice treated.	Number of mice which became negative.	Time taken to become negative. Hours.	Length of time mice remained negative. Days.	Remarks.
Normal strain	0·1	20 to +	5	0	...	...	Infection controlled.
	0·15	2 to +	10	2	12-24	2 mice : 1	Infection controlled.
	0·175	20 to ++	4	0	...	...	Dose lethal.
Atoxyl-Resistant strain	0·15	1/2 to 10	5	1	3-6	1 mouse : 1	Infection controlled.
	0·175	+ to ++	4	0	...	...	Dose lethal.
	0·2	5 to 15	5	1	6-18	1 mouse : 1	Dose lethal.
Acriflavine-resistant	0·15	2 to 10	5	2	12-36	2 mice : 1	Infection controlled.

Table XVI shows that for sodium arsinite—

Lethal dose = 0·175 mgm.

For infections due to the normal strain—

M.E.D. = 0·15+ mgm.

M.C.D. not obtainable.

For infections due to the resistant strains—

M.E.D. = 0·15+ mgm.

∴ Resistant factor = 1.

TABLE XVII

STIBENYL in the treatment of mice infected with *T. rhodesiense* (normal, atoxyl- and acriflavine-resistant strains)

Strain.	Dose per 20 gm. mouse. mgm.	Degree of infection of mouse when treated. Parasites per field. (1/6 obj. 4 oc.)	Number of mice treated.	Number of mice which became negative.	Time taken to become negative. Hours.	Length of time mice remained negative. Days.	Remarks.
Normal strain	0·625	+	2	0			
	1·25	20 to +	3	1	72-96	1 mouse : 1	
	2·5	20 to ++	2	0			
	3·75	1/3 to 5	5	3	60-96	2 mice : 2 1 mouse : 3	
	5·0	1/10 to ++	8	8	12-36	1 mouse : 6 1 mouse : 7† 1 mouse : 8 2 mice : 11 2 mice : cured	
	7·5	1 to 10	5	5	12-48	2 mice : 9 1 mouse : 12 1 mouse : 16 1 mouse : cured	
	10·0	1/20 to 1/2	5	5	8-12	5 mice : cured	
	15·0	1/10 to 4	5	5	6-12	5 mice : cured	
Atoxyl-resistant strain	5·0	5 to ++	3	0			
	7·5	1/5 to 4	5	0			
	10·0	1 to 3	5	0			
	20·0	1/2 to 20	5	3	12-96	1 mouse : 2† 1 mouse : 3 1 mouse : 8	
	30·0	3 to +	5	0	...	...	Dose lethal, all mice dead in 6-24 hours.
Acriflavine-resistant strain	20·0	1/2 to 5	5	3	12-72	1 mouse : 2† 1 mouse : 17 1 mouse : cured	
	30·0	1/10 to 8	5	0	...	...	Dose lethal, all mice dead in 6-24 hours.

† Mouse died without relapse.

Table XVII shows that for stibenyl—

Lethal dose = 25 to 30 mgm.

For infections due to the normal strain—

M.E.D. = 4 mgm.

M.C.D. = 10 mgm.

For infections due to resistant strains—

M.E.D. = 20+ mgm.

∴ Resistant factor = 5+

TABLE XVIII

STIBOSAN in the treatment of mice infected with *T. rhodesiense* (normal, atoxyl-, and acriflavine-resistant strains)

Strain.	Dose per 20 gm. mouse. mgm.	Degree of infection of mouse when treated. Parasites per field. (1/6 obj. 4 oc.)	Number of mice treated.	Number of mice which became negative.	Time taken to become negative. Hours.	Length of time mice remained negative. Days.	Remarks.
Normal strain	1·25	1/10 to ++	12	2	12-48	2 mice : cured	
	2·5	1/20 to ++	11	9	24-96	3 mice : 4 1 mouse : 6 2 mice : 7 3 mice : cured	
	3·75	1/5 to 10	5	5	48-72	1 mouse : 13 1 mouse : 14 3 mice : cured	
	5·0	1 to 5	5	5	12-18	1 mouse : 21 4 mice : cured	
Atoxyl-resistant strain	1·25	1/10 to ++	8	1	72-96	1 mouse : 4	Infection markedly controlled.
	2·5	1/10 to ++	12	3	12-96	1 mouse : 4 1 mouse : 5† 1 mouse : 18	
	5·0	1/20 to +	15	6	96-168	4 mice : 1 2 mice : 2	
	7·5	3 to +	5	5	96-120	5 mice : 3	
	10·0	2 to 5	5	5	12-48	1 mouse : 2† 1 mouse : 8† 1 mouse : 10 1 mouse : 29 1 mouse : cured	
	15·0	1/3 to 5	5	5	4-12	2 mice : 1† 1 mouse : 3† 1 mouse : 17† 1 mouse : cured	Dose lethal.
Acriflavine-resistant strain	2·5	1/50 to 1/2	5	0	...	...	Infection markedly controlled.
	5·0	1/20 to 5	10	4	48-144	1 mouse : 1 1 mouse : 2 1 mouse : 3 1 mouse : 7	
	7·5	1/10 to 10	4	3	48-96	1 mouse : 3 1 mouse : 9 1 mouse : cured	
	10·0	1 to 20	5	5	12-48	1 mouse : 9 1 mouse : 10 1 mouse : 17 2 mice : cured	
	15·0	1/5 to 5	5	5	4-12	1 mouse : 1† 3 mice : 2† 1 mouse : 7†	Dose lethal.

† Mouse died without relapse.

Table XVIII shows that for stibosan—

Lethal dose = 15 mgm.

For infections due to the normal strain—

M.E.D. = 2·5 mgm.

M.C.D. = 5 mgm.

For infections due to the resistant strains—

M.E.D. = 7·5 mgm.

M.C.D. = 10+ mgm.

∴ Resistant factor = 3.

TABLE XIX

TARTAR EMETIC in the treatment of mice infected with *T. rhodesiense* (normal, atoxyl- and acriflavine-resistant strains)

Strain.	Dose per 20 gm. mouse. mgm.	Degree of infection of mouse when treated. Parasites per field. (1/6 obj. 4 oc.)	Number of mice treated.	Number of mice which became negative	Time taken to become negative. Hours.	Length of time mice remained negative. Days.	Remarks.
Normal strain	0·3	1/5 to +	10	7	3-9	4 mice : 1 1 mouse : 2 1 mouse : 3 1 mouse : 4	
	0·4	1/5 to ++	10	9	2-24	2 mice : 1 3 mice : 2 1 mouse : 3 1 mouse : 4 1 mouse : 7 1 mouse : 8	
	0·5	1/15 to 1	5	5	1-3	3 mice : 3 2 mice : 4	
	0·75	1/20 to 20	11	11	1-2	1 mouse : 2† 1 mouse : 2 1 mouse : 3 2 mice : 7 4 mice : 9 2 mice : 10	
	1·0	3 to 10	5	5	1-2	1 mouse : 7 1 mouse : 8 1 mouse : 8† 1 mouse : 10 1 mouse : 17	
	1·5	—	2	...	...	...	Dose lethal, both mice dead in 12 hours.
	2·0	—	2	...	...	...	Dose lethal, both mice dead in 12 hours.
Atoxyl-resistant strain	0·3	1 to 15	8	0	...	...	Infection controlled.
	0·4	1 to ++	10	9	3-6	6 mice : 3 1 mouse : 4 2 mice : 5	
	0·5	1/10 to 5	8	7	2-3	2 mice : 2 1 mouse : 4 2 mice : 5 2 mice : 6	
	0·75	1/3 to ++	11	10	1-3	2 mice : 1† 1 mouse : 3 3 mice : 7 3 mice : 8 1 mouse : 11	
	1·0	1 to +	7	7	1-2	1 mouse : 1† 2 mice : 7 3 mice : 9 1 mouse : 12†	

TABLE XIX—*continued*

TARTAR EMETIC in the treatment of mice infected with *T. rhodesiense* (normal, atoxyl and acriflavine-resistant strains)

Strain.	Dose per 20 gm. mouse. mgm.	Degree of infection of mouse when treated. Parasites per field. (1/6 obj. 4 oc.)	Number of mice treated.	Number of mice which became negative.	Time taken to become negative. Hours.	Length of time mice remained negative. Days.	Remarks.
Acriflavine-resistant strain	0·3	1/20 to 3	5	2	6-12	2 mice : 1	Infection controlled.
	0·4	1/10 to 3	5	5	2-3	1 mouse : 1 1 mouse : 2 3 mice : 5	
	0·5	1/5 to 3	5	5	1-3	1 mouse : 2 2 mice : 3 2 mice : 5	
	0·75	1/10 to 20	7	7	1-3	1 mouse : 2† 1 mouse : 5 2 mice : 7 2 mice : 9 1 mouse : 14	
	1·0	1/3 to 5	5	5	1-2	1 mouse : 7 4 mice : 11	

† Mouse died without relapse.

Table XIX shows that for tartar emetic—
Lethal dose = 1·5 mgm.
For infections due to the normal strain—
M.E.D. = 0·4 mgm.
M.C.D. not obtainable
For infections due to the resistant strains—
M.E.D. = 0·4 mgm.

∴ Resistant factor = 1

TABLE XX

ACRIFLAVINE in the treatment of mice infected with *T. rhodesiense* (normal, atoxyl- and acriflavine-resistant strains)

Strain.	Dose per 20 gm. mouse. mgm.	Degree of infection of mouse when treated. Parasites per field (1/6 obj. 4 oc.)	Number of mice treated.	Number of mice which became negative.	Time taken to become negative. Hours.	Length of time mice remained negative. Days.	Remarks.
Normal strain	0·1	1/3 to 5	5	5	96-120	1 mouse : 1 1 mouse : 3 3 mice : 4	
	0·2	1 to 10	4	4	12-72	1 mouse : 4 1 mouse : 13 1 mouse : 18 1 mouse : cured	
	0·3	1/5 to +	9	8	6-24	1 mouse : 2† 2 mice : 7† 1 mouse : 14† 1 mouse : 25 3 mice : cured	
	0·4	15 to +	3	3	12-24	3 mice : cured	
Atoxyl-resistant strain	0·4	1/3 to 15	5	0	...	...	Infection uninfluenced.
	0·5	1 to 15	5	0	...	...	Dose lethal, 3 mice dead in 3-12 hours.
	1·0	1/2 to 20	5	0	...	...	Dose lethal, all mice dead in 6-12 hours.
Acriflavine-resistant strain	0·4	8 to ++	5	0	...	...	Infection uninfluenced.

† Mouse died without relapse.

Table XX shows that for acriflavine—
Lethal dose = 0·5 mgm.
For infections due to the normal strain—
M.E.D. = 0·1 mgm.
M.C.D. = 0·4 mgm.
For infections due to the resistant strains—
M.E.D. = 0·4++ mgm.

∴ Resistant factor = 4++

TABLE XXI

BAYER 205 in the treatment of mice infected with *T. rhodesiense* (normal, atoxyl- and acriflavine-resistant strains)

Strain.	Dose per 20 gm. mouse. mgm.	Degree of infection of mouse when treated. Parasites per field. (1/6 obj. 4 oc.)	Number of mice treated.	Number of mice which became negative.	Time taken to become negative. Hours.	Length of time mice remained negative. Days.	Remarks.
Normal strain.	0·0125	1/2 to 20	5	2	36	1 mouse : 1 1 mouse : 4	
	0·025	1/2 to 10	4	3	36	1 mouse : 4 1 mouse : 5 1 mouse : 10	
	0·05	1/10 to 5	4	4	18-36	2 mice : 20 2 mice : cured	
	0·075	1/30 to 5	10	10	18-60	1 mouse : 13 1 mouse : 13† 8 mice : cured	
	0·1	1 to 20	2	2	24	2 mice : cured	
	10·0	...	5	...	...	...	All mice survived.
	15·0	...	5	...	...	...	Dose lethal; 3 mice dead in 5 days.
	20·0	...	5	...	...	...	Dose lethal; 5 mice dead in 4 to 24 hours.
Atoxyl-resistant strain.	0·0125	1 5 to 3	5	1	60	1 mouse : 3	
	0·025	1 5	2	2	18-36	1 mouse : 8 1 mouse : 9	
	0·05	1 10 to 1 3	2	2	18	1 mouse : 13 1 mouse : cured	
	0·075	1 30 to 2	10	10	18	1 mouse : 14† 1 mouse : 25† 1 mouse : 28 7 mice : cured	
	0·1	1 15 to 1 3	2	2	18	2 mice : cured	
	0·2	5	2	2	24-36	2 mice : cured	
Acriflavine-resistant strian.	0·0125	1 to ++	5	1	48	1 mouse : 4	
	0·025	10	3	3	36	2 mice : 5 1 mouse : 18	
	0·05	3 to 10	3	3	18-36	1 mouse : 10 1 mouse : 29 1 mouse : cured	
	0·075	1 50 to ++	9	9	18-60	1 mouse : 9 1 mouse : 13 1 mouse : 15 1 mouse : 24 5 mice : cured	
	0·1	10 to 20	2	2	36	2 mice : cured	

† Mouse died without relapse

Table XXI shows that for Bayer 205—

Lethal dose = 15 mgm.

For infections due to the normal strain—

M.E.D. = 0·025 mgm.

M.C.D. = 0·075 mgm.

For infections due to the resistant strains—

M.E.D. = 0·025 mgm.

M.C.D. = 0·075 mgm.

∴ Resistant factor = 1

TABLE XXII

Giving the lethal doses of the various compounds, their M.E.D. and M.C.D. for the normal and resistant strains, and their minimal trypanocidal concentrations for the same strains *in vitro*.

Drug	*In vivo* experiments					*In vitro* experiments		Resistant factors*	
	Lethal dose mgm. per 20 gm. mouse	M.E.D.		M.C.D.		Concentration necessary to destroy trypanosomes within 24 hours at 37° C.		*In vivo*	*In vitro*
		Normal strain. mgm.	Resistant strains. mgm.	Normal strain. mgm.	Resistant strains. mgm.	Normal strain	Resistant strains		
Atoxyl	10—15	2	10+	6	—	1 : 1,600	1 : 400	5+	2—4
Reduced atoxyl thioglycollate	0·15	0·075	0·15++	—	—	1 : 102,400,000	1 : 25,600,000	2++	4—8
Sod. 4-hydroxy-phenyl-arsonate	10—15	1·5	10++	5	—	1 : 6,400	1 : 1,600	7++	4
4-hydroxy-phenyl-arsenoxide	0·2	0·0375	0·15++	0·1+	—	1 : 409,600,000	1 : 25,600,000	4++	16
Arsacetin	50	4	40+	5	—	1 : 1,600	1 : 800	10+	2
Red. arsacetin... ...	0·375	0·025	0·375++	0·1	—	1 : 102,400,000	1 : 3,200,000	15++	32
Tryparsamide...	100	6	100+	20	—	1 : 1,600	1 : 1,600	16+	1
Red. tryparsamide thioglycollate	3	0·03-0·04	2++	0·5	—	1 : 102,400,000	1 : 200,000	60++	512
Sod. stovarsol ..	50—75	30	50+	—	—	1 : 1,600	1 : 800	2+	2
Red. stovarsol thioglycollate	1	0·15	0·8+	0·4	—	1 : 6,400,000	1 : 400,000	6+	16
Halarsol ...	1	0·02	0·75++	0·1	—	1 : 102,400,000	1 : 3,200,000	37·5++	32—64
Halarsol thioglycollate	1·5	0·03	1·2++	0·1	—	1 : 102,400,000	1 : 3,200,000	40++	32—64
Novarsenobillon .	5	0·125	4+	1	—	1 : 51,200,000	1 : 1,600,000	32+	32—64
Disodium 4-glycine-phenyl-arsonate	60	60	60	—	—	1 : 400	1 : 200 to 1 : 400	1	1—2
Arseno-phenyl-glycine	2	0·5	0·5-0·6	1	1·5+	1 : 3,200,000	1 : 1,600,000	1—2	2
Sodium arsinite ..	0·175	0·15+	0·15+	—	—	1 : 3,200,000	1 : 3,200,000	1	1
Stibenyl	25—30	4	20+	10	—	1 : 12,800	1 : 12,800	5+	1
Stibosan	15	2·5	7·5	5	10+	1 : 12,800	1 : 12,800	3	1
Tartar emetic... ...	1·5	0·4	0·4	—	—	1 : 6,400,000	1 : 6,400,000	1	1
Acriflavine	0·5	0·1	0·4++	0·4	—	1 : 25,600.000	1 : 1,600,000	4++	8—16
Bayer 205	15	0·025	0·025	0·075	0·075	1 : 1,600	1 : 1,600	1	1

*In *in vivo* experiments Resistant factor $= \frac{\text{M.E.D. for resistant infections}}{\text{M.E.D. for normal infection}}$

In *in vitro* experiments Resistant factor $= \frac{\text{trypanocidal concentration for resistant strains}}{\text{trypanocidal concentration for normal strain}}$

arsonate, and tartar emetic. In the case of the two resistant strains—atoxyl- and acriflavine-resistant—both of which invariably responded in exactly the same way, the M.E.D. exceeded the lethal dose in all but five of the drugs, viz., disodium 4-glycine-phenyl-arsonate, arsenophenylglycine, stibosan, tartar emetic and Bayer 205, whilst the M.C.D. exceeded the lethal dose in every case but that of Bayer 205.

Notwithstanding these limitations, the broad fact clearly emerges that the resistant strains show, in these *in vivo* experiments, degrees of resistance to the various drugs comparable to those which we have previously found them to exhibit in the *in vitro* work.

If we confine ourselves in the first instance to consideration of those compounds which previous work (1930) has shown to be actively trypanocidal *in vitro*, we find that to such drugs as arsenophenylglycine and to the non-phenyl compounds of arsenic and antimony—sodium arsinite and tartar emetic—the resistant strains were almost as insensitive as was the normal strain ; whereas to reduced tryparsamide, novarsenobillon, halarsol and reduced arsacetin the resistant strains exhibited a relatively very high degree of resistance. The M.E.D. of arsenophenylglycine, sodium arsinite and tartar emetic for the resistant strains were practically the same as for the normal strain, and hence the ' resistant factor ' for each of them is approximately 1. In the case, however, of such compounds as reduced tryparsamide, halarsol and reduced arsacetin, we were unable to discover the M.E.D. for the resistant infections because in no instance did the lethal dose or sub-lethal dose exert the slightest influence on these infections. Nevertheless, we can at least state of these drugs that their respective minimum effective doses for the resistant infections are greater than their respective lethal doses ; and we can represent the M.E.D. of reduced tryparsamide for the resistant infections by the expression 2 ++ mgm., that of halarsol as 0·75 ++ mgm., and that of reduced arsacetin as 0·375 ++ mgm. Such information enables us to express the resistant factor for reduced tryparsamide as $\frac{2\ ++}{0{\cdot}03\text{-}0{\cdot}04} = 60\ ++$, for halarsol as $\frac{0{\cdot}75++}{0{\cdot}02} = 37{\cdot}5++$ and for reduced arsacetin as $\frac{0{\cdot}375++}{0{\cdot}025} = 15++$.

Turning to novarsenobillon we find that sub-lethal doses of 4 mgm., whilst failing to clear the peripheral blood of mice infected with the resistant strains, nevertheless exerted a definitely inhibiting

influence on the course of the infection. Apparently, therefore, the M.E.D. of this drug for the resistant strains was only slightly greater than the lethal dose ; and we consequently record the M.E.D. of novarsenobillon for the resistant strain as 4 + mgm. and the resistant factor as $\frac{4+}{0{\cdot}125} = 32+$.

If we contrast the figures, relating to the trivalent compounds, given in the last two columns of Table XXII, we find that, allowing for the limitations imposed by the lethal doses of the various drugs, the ' resistant factors ' obtained from the *in vivo* experiments exhibit a broad correspondence with those obtained from the previous *in vitro* experiments. Thus for 4-hydroxy-phenyl-arsenoxide the resistant factors obtained were *in vivo* 4 ++ and *in vitro* 16 ; for reduced arsacetin they were respectively 15 ++ and 32 ; for reduced tryparsamide thioglycollate 60 ++ and 512 ; for reduced stovarsol thioglycollate 6 + and 16 ; for halarsol 37·5 ++ and 32 to 64 ; for halarsol thioglycollate 40 ++ and 32 to 64 ; for novarsenobillon 32 + and 32 to 64 ; for arsenophenylglycine 1 to 2 and 2 ; for sodium arsinite 1 and 1, and for tartar emetic 1 and 1.

The experiments with the pentavalent compounds, atoxyl, sodium 4-hydroxy-phenyl-arsonate, arsacetin, tryparsamide, sodium stovarsol, disodium 4-glycine-phenyl-arsonate, stibenyl and stibosan do not allow of such a comparison because, as we have already pointed out in an earlier paper, and as can be seen by reference to the appropriate column in Table XXII, these pentavalent compounds are practically without trypanocidal action *in vitro*, being in fact definitely less active than are aniline or phenol. It is, however, important to note that in the *in vivo* experiments the resistant infections exhibited very varying degrees of resistance to the different compounds, e.g., to some of them such as arsacetin and tryparsamide this resistance was very great, but to disodium 4-glycine-phenyl-arsonate it was very slight or absent. This observation seems to have an important significance to which we shall refer later.

The facts that the resistant strains exhibited very different degrees of resistance to the various aromatic compounds of arsenic (both trivalent and pentavalent), and that they were no more resistant to sodium arsinite than was the normal strain, confirm the conclusion reached from similar observations made *in vitro* that the resistance

of the atoxyl- and acriflavine-resistant strains is not a resistance to arsenic but is dependent on the structure of the substituted phenyl radical in the various aromatic compounds of arsenic. Further support for this contention is afforded by the reaction of the resistant strains to the various compounds of antimony. To the aromatic compounds they exhibited definite resistance but to tartar emetic they were just as sensitive as was the normal strain.

In conclusion, reference might be made to several facts which support the hypothesis that in the case of the arsenoxides, the arsenobenzols and the non-phenyl trivalent compounds of arsenic and antimony, the therapeutic effect is due to the direct action of the unchanged drugs on the trypanosomes, whereas in the case of the pentavalent aromatic compounds it occurs only after reduction of the drugs to their corresponding trivalent forms.

As can be seen from the appropriate tables, the M.E.D. and M.C.D. of the arsenoxides are very minute as compared with the M.E.D. and M.C.D. of the corresponding pentavalent compounds. For example, the M.E.D. of reduced arsacetin is 0·025 mgm. and the M.C.D. 0·1 mgm., whereas those of arsacetin are respectively 4 mgm. and 5 mgm.; for reduced tryparsamide the figures are 0·035 mgm. and 0·5 mgm., and for tryparsamide 6 mgm. and 20 mgm.; and for reduced stovarsol they are 0·15 mgm. and 0·4 mgm., and for stovarsol 30 mgm. and 40 + mgm.

Furthermore, the arsenoxides, even though given in such relatively minute doses, clear the blood of infected mice definitely more quickly than do the much greater doses of the pentavalent compounds. For instance, twice the M.E.D. of reduced tryparsamide, i.e., 0·06 mgm., produced peripheral sterilization of infected mice within two to four hours, whereas twice the M.E.D. of tryparsamide, i.e., 12 mgm., requires six to twelve hours to clear the blood of parasites.

Moreover, it is not without interest to note (Table XXII) that, broadly speaking, amongst the trivalent compounds those which we had found to be most actively trypanocidal *in vitro* proved to be most active *in vivo*, and conversely those which were less active *in vitro* were less active *in vivo*. For example, when the normal strain is used, reduced atoxyl thioglycollate, 4-hydroxy-phenyl-arsenoxide, reduced arsacetin, reduced tryparsamide thioglycollate, halarsol, and halarsol thioglycollate, all of which are powerfully trypanocidal

in vitro even in such feeble concentrations as 1 : 100 millions, were found *in vivo* to have minimum effective doses varying from 0·02 to 0·075 mgm. ; whereas the minimum effective doses of arsenophenylglycine and tartar emetic, which *in vitro* are trypanocidal in concentrations of about one in three to six millions, are about 0·4 to 0·5 mgm. In fact, both for the normal and resistant strains there is a close parallelism between the trypanocidal power of the arsenoxides, arsenobenzols and non-phenyl trivalent compounds of arsenic and antimony *in vitro*, and their minimum effective doses.

Although then, there seems good reason for believing that the therapeutic activity of the arsenoxides, arsenobenzols, and the non-phenyl trivalent compounds of arsenic and antimony, is due to the direct trypanocidal action of the unchanged drugs, the mechanism of the therapeutic action of the pentavalent compounds is not quite so clear. It seems obvious that in their case the therapeutic activity cannot be explained by direct action. *In vitro* their trypanocidal activity is exceedingly slight, being in fact less than that of aniline or phenol. Their minimum effective doses *in vivo* are, however, although very much greater than those of the corresponding reduced products, much too small to render tenable the view that their therapeutic effect is due to the trypanocidal action of the unchanged drugs. Whilst it must be admitted that the proof that they owe their therapeutic activity to their reduction in the body of the host is not yet complete, the results obtained by us in the study of their action on both the normal and resistant infections are quite in harmony with the hypothesis. One of the most important points suggesting that the hypothesis is correct is the effect of the pentavalent compounds on the resistant infections. *In vitro* the resistant strains are but slightly more resistant to them than is the normal strain, but *in vivo* the resistant infections are seen to exhibit very considerable resistance to atoxyl, arsacetin, tryparsamide and stovarsol, but practically none to disodium 4-glycine-phenyl-arsonate. In short, whilst the resistant factor obtained from the *in vitro* work is in each case only 1 to 2, the factors obtained from the *in vivo* work are : for atoxyl 5 +, arsacetin 10 +, tryparsamide 16 +, stovarsol 2 +, but for disodium 4-glycine-phenyl-arsonate only 1. Thus, in each instance there is a definite tendency for the resistant factor to approximate to that of the corresponding reduced product.

SUMMARY

1. An attempt has been made to confirm by experiments *in vivo* the conclusions reached in a previous paper from observations *in vitro* regarding the action of a considerable number of arsenical and antimonial compounds on *T. rhodesiense* and on atoxyl- and acriflavine-resistant varieties of this parasite.

2. The minimum effective dose (M.E.D.) and the minimum curative dose (M.C.D.) were determined for each drug in respect of infections in mice produced by the normal strain.

3. No differences could be detected between the reactions of the two resistant strains to the various drugs. Owing to the fact that with most of the drugs these infections resisted doses up to and including the lethal dose, it proved impossible except in the case of disodium-4-glycine-phenyl-arsonate, arsenophenylglycine, stibosan, tartar emetic and Bayer 205, to determine exactly even the minimum effective dose for these strains ; and only in the case of Bayer 205 could the minimum curative dose be ascertained.

4. Notwithstanding the limitations which this fact has necessarily imposed on these *in vivo* experiments, we have been enabled to obtain sufficient information to confirm the main conclusions reached from our *in vitro* work. To the various arsenoxides and arsenobenzols, the resistant strains exhibited different degrees of resistance. For example, in the case of arsenophenylglycine, the M.E.D. for the resistant infections was practically the same as that for the normal infection, whereas in the case of reduced tryparsamide thioglycollate the M.E.D. for the resistant strains was undoubtedly considerably more than sixty times that for the normal strain.

5. This fact, together with the observation that the resistant infections were no more resistant to sodium arsinite than was the normal strain, indicates that the resistance of the atoxyl- and acriflavine-resistant strains is not a resistance to arsenic but is dependent on the structure of the substituted phenyl radical in the various aromatic compounds of arsenic and antimony.

6. This contention is supported by the fact that whilst the resistant strains proved to be definitely resistant both to stibenyl

and to stibosan, they were just as susceptible to tartar emetic as was the normal strain.

7. It is interesting to note that the resistant strains responded to Bayer 205 in almost exactly the same manner as did the normal strain.

8. The facts that the M.E.D. and M.C.D. of the arsenoxides and of the arsenobenzols are exceedingly small as compared with the M.E.D. and M.C.D. of the corresponding pentavalent compounds, and the sterilization of the peripheral circulation is produced more rapidly by the arsenoxides and arsenobenzols than by the pentavalent compounds, support the hypothesis that therapeutic activity in the case of the former compounds is due to the direct trypanocidal effect of the unchanged drugs and in the case of the latter to an indirect action dependent on their reduction, in the body of the host, to the corresponding trivalent forms.

9. Further strong support to this hypothesis is provided by the observation that both in the case of the normal strain and in that of the resistant strains, there was found to be a close parallelism between the minimum effective doses of the various drugs *in vivo* and their minimum trypanocidal concentrations *in vitro*. In fact, all strains responded in the *in vivo* experiments in precisely the manner in which the previous *in vitro* experiments had indicated that they would respond, on the assumption that the therapeutic activity of the arsenoxides, arsenobenzols, sodium arsinite and tartar emetic depended on the direct trypanocidal power of the unaltered drugs, whilst that of the pentavalent compounds depended on their reduction in the body of the host into their corresponding trivalent forms.

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STUDIES IN CHEMOTHERAPY*

V.—PRELIMINARY CONTRIBUTION ON THE NATURE OF DRUG RESISTANCE

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In 1907 Franke and Roehl, working in Ehrlich's laboratory, recorded a very interesting observation (Ehrlich, 1907). They found that the feeding of nagana-infected mice with parafuchsin caused the disappearance of trypanosomes from the peripheral blood; after a week or two, however, the parasites re-appeared but further administration of the drug caused them to disappear again. The process could be repeated, but not indefinitely, as after a time it was noticed that the parafuchsin feeds began to produce less and less effect; after each successive administration of the drug the time of banishment of the parasites from the blood of the mouse became shorter and shorter until finally a period was reached when the drug entirely failed to influence the parasites. On transference of the parasites to normal mice they were found to be still uninfluenced by parafuchsin, and it became evident that they had in fact acquired a heightened resistance to the drug. This fundamental observation was quickly followed by the discovery that strains of trypanosomes resistant to other drugs could be developed.

The practical importance of the capacity of trypanosomal infections to become drug-resistant, or drug-fast, for the treatment of these infections in man and stock was at once recognized by Ehrlich, and, during the last twenty years, the whole question in its various aspects has been the subject of an enormous amount of work. It is not our intention at the present time to discuss the phenomenon of drug resistance in any detail, or to attempt to

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orientate ourselves through the mass of observations—true and false—which its investigation has called forth, but merely to record briefly a number of experiments which appear to us to throw light on one of the many important and difficult problems which the subject of drug-resistance presents.

According to Ehrlich (1908) resistance is due to a reduction of the avidity of the specific chemo-receptors for the drug to which the strain has been made resistant. Broadly speaking this hypothesis held the field unchallenged until 1923 when it was criticized by Dale (1923).

The following year Voegtlin, Dyer and Miller (1924) state that they agree with Dale in almost every criticism he offers against the validity of the chemoreceptor theory. They write ' in fact, it was just this realization of the total inadequacy of this theory, which several years ago led us to take up the study of chemotherapy.' After defining arsenic resistance as a relative term relating the resistance of one strain to that of another strain (*a*) in terms of the difference in time required to kill each strain when both strains are exposed to the same concentration of the drug, or (*b*) in terms of the minimum lethal drug concentration for both strains, Voegtlin, Dyer and Miller enquire ' what then is the cause of this greater length of time required to kill the more resistant strain ? ' They conceive of two possible explanations : The strain with the higher resistance may owe its greater resistance either to reduction in permeability of the parasites for the drug, or to a fortification of the natural physicochemical defence mechanism of the protoplasm against the toxic action of the drug. The former of these two hypotheses is abandoned because no experimental evidence is available in support of it. There are, however, in the opinion of Voegtlin, Dyer and Miller some very suggestive considerations in support of the second possibility. Voegtlin, Dyer and Leonard (1923) had reached the conclusion that the toxic action of arsenoxide (R. As = O) is essentially due to a condensation with the sulphydryl compounds of protoplasm. This interferes with the proper functioning of the system regulating cell respiration of which the sulphur compounds are an integral part. If every sulphydryl compound has been withdrawn by the arsenic (R. As = O) from active participation in this system, the cell is asphyxiated and dies. The authors consider

that whether a trypanosome will survive or die, or how long it will survive exposure to a given concentration of arsenoxide, may depend on whether the system of which the sulphur compounds are an integral part is so adjusted that it contains a sufficient excess (above the physiological requirement) of sylphydryl compounds available for combination with arsenic. According to this view variations in the arsenic resistance of different strains would depend essentially on the relative excess of sulphydryl compounds above that of the physiological requirement of the different strains.

We are not in this paper concerned with a critical examination of the numerous observations upon which Voegtlin and his colleagues have erected this ingenious hypothesis, but merely to submit the hypothesis itself to what appears to us to be a crucial test. According to Voegtlin's hypothesis—if we understand it correctly—a strain of trypanosomes exhibiting a pronounced degree of drug resistance should, on exposure to a given concentration of the arsenical to which it is resistant, be capable, in virtue of its excess of sulphydryl compounds, of absorbing more of the arsenical than is the homologous normal strain ; whilst according to Ehrlich's theory, which postulates that resistance on the part of a strain is due to a reduction of the avidity of its specific chemoreceptors, the resistant strain should be capable of absorbing less of the arsenical, to which it is resistant, than is the normal strain. It was with the object of obtaining an answer to this question that we performed a number of experiments of the following general character.

Large numbers of trypanosomes—normal and resistant—were suspended for an hour at 37° C. in the usual nutrient medium, consisting of equal parts of rabbit serum (heated to 62°-64°C. for 30 minutes) and Ringer-glucose solution, containing a certain concentration of a drug to which the resistant strain was resistant. Particular care was taken so to regulate the amount of drug added to the nutrient medium, and the number of trypanosomes employed, that there was always an excess of the latter, with the result that the concentration of drug was insufficient to injure the parasites even of the normal strain within so short a period as one hour. The trypanosomes were then removed by centrifugalization : the supernatant fluids were tested for the presence of drug by the biological method of ascertaining their trypanocidal action on fresh

parasites of the normal strain ; and the trypanosomes themselves, after being washed thoroughly, were incubated in fresh nutrient medium for twenty-four hours in order to make sure that they had not absorbed a lethal quantity of the drug.

The protocol of one of these experiments is given below :—

EXPERIMENT :—The heart blood of a rabbit was allowed to clot at 37° C. for two hours, and the serum removed and centrifuged at high speed to separate all cellular elements. The serum was then heated at 62°-64° C. for 30 minutes and added to an equal quantity of Ringer-glucose solution* in order to form the ' nutrient medium.'

A mouse, heavily infected with *T. rhodesiense* (normal strain), was killed and its blood added to citrated Ringer-glucose solution. The citrated blood was then centrifuged at low speed and the supernatant fluid, containing the trypanosomes, withdrawn from the deposit of red corpuscles. After repeating this process to remove the remaining red cells, the supernatant fluid was centrifuged at high speed to throw down the trypanosomes, which were then washed with a large volume of ' nutrient medium ', again thrown down in the high speed centrifuge, and finally taken up in 0·1 c.c. of ' nutrient medium.' A concentrated suspension of the homologous atoxyl-resistant strain was prepared in an exactly similar manner.

Small volumes of these concentrated trypanosome suspensions were then added to tubes containing 1 c.c. of nutrient medium, with or without reduced tryparsamide thioglycollate, as is shown in the following table, and incubated at 37° C. for one hour with frequent shaking to prevent sedimentation of great masses of parasites.

Tube	Nutrient Medium	Concentration of drug—reduced tryparsamide thioglycollate—in nutrient medium	Concentrated suspension of trypansosomes	No. of trypanosomes per 256 squares of the haemocytometer scale
A	1 c.c.	1 : 12,800,000	0·05 c.c. normal	15,000
B	1 c.c.	...	0·05 c.c. normal	15,000
C	1 c.c.	1 : 12,800,000	0·05 c.c. resistant	15,000
D	1 c.c.	...	0·05 c.c. resistant	15,000
E	1 c.c.	1 : 12,800,000	...	...

After an hour's sojourn in the incubator all the tubes were removed, and Tubes A to D were centrifuged at high speed to throw down the trypanosomes. The supernatant fluids were removed and tested biologically for the presence of reduced tryparsamide thioglycollate ; this was done by the incubation of relatively small numbers of fresh trypanosomes of the normal strain in the undiluted fluids and in various dilutions of them. The deposited trypanosomes were washed thrice in large volumes of fresh nutrient medium to remove all traces of the drug, and finally suspensions of moderate strength were made in this medium and incubated at 37° C. for 24 hours. The results are shown in the following table :—

* Sodium chloride 0·9 gm. Potassium chloride 0·025 gm. Calcium chloride 0·02 gm. Glucose 0·2 gm. Water 100·0 c.c.

Tube	Medium	Concentration of drug originally present	Trypanosomes	Number of living trypanosomes per 256 squares of the haemocytometer scale			
				Start	4 hours	8 hours	24 hours
1	Supernatant fluid of Tube A + glucose to 0·1%	1 : 12,800,000	Fresh normal		136	98	0
2	" 1 pt. + nutrient medium 1 pt.	1 : 25,600,000	"		...	92	61
3	" 1 pt. + " " 3 pts.	1 : 51,200,000	"		...	...	110
4	" 1 pt. + " " 7 pts.	1 : 102,400,000	"		...	...	100
5	Supernatant fluid of Tube B + glucose to 0·1%	...	"		138	116	0
6	" 1 pt. + nutrient medium 1 pt.	...	"		110	108	60
7	Supernatant fluid of Tube C + glucose to 0·1%	1 : 12,800,000	"		25	0	...
8	" 1 pt. + nutrient medium 1 pt.	1 : 25,600,000	"		70	8	0
9	" 1 pt. + " " 3 pts.	1 : 51,200,000	"		...	86	0
10	" 1 pt. + " " 7 pts.	1 : 102,400,000	"		...	108	24
11	" 1 pt. + " " 15 pts.	1 : 204,800,000	"	100	...	...	103
12	" 1 pt. + " " 31 pts.	1 : 409,600,000	"		...	...	110
13	Supernatant fluid of Tube D + glucose to 0·1%	...	"		120	130	7
14	" 1 pt. + nutrient medium 1 pt.	...	"		112	114	76
15	Supernatant fluid of Tube E undiluted	1 : 12,800,000	"		0	...	...
16	" 1 pt. + nutrient medium 1 pt.	1 : 25,600,000	"		37	0	...
17	" 1 pt. + " " 3 pts.	1 : 51,200,000	"		86	1	...
18	" 1 pt. + " " 7 pts.	1 : 102,400,000	"		...	100	0
19	" 1 pt. + " " 15 pts.	1 : 204,800,000	"		140	...	92
20	" 1 pt. + " " 31 pts.	1 : 409,600,000	"		...	...	122
21	Nutrient medium + freshly added Reduced tryparsamide thioglycollate	1 : 12,800,000	Fresh normal		0	...	...
22		1 : 25,600,000	"		5	0	...
23		1 : 51,200,000	"		58	0	...
24		1 : 102,400,000	"	100	90	43	0
25		1 : 204,800,000	"		...	82	23
26		1 : 409,600,000	"		...	...	112
27	Nutrient medium (Control)	...	"		...	130	87
28	Nutrient medium + freshly added Reduced tryparsamide thioglycollate	1 : 25,000	Fresh resistant		0	...	...
29		1 : 50,000	"		40	0	...
30		1 : 100,000	"		88	1	...
31		1 : 200,000	"		...	66	0
32		1 : 400,000	"	100	...	112	0
33		1 : 800,000	"		...	...	34
34		1 : 1,600,000	"		...	...	58
35	Nutrient medium (Control)	...	"		...	90	88
36	Nutrient medium	...	Tube A	110	100	88	86
37	" "	...	" B	110	92	122	90
38	" "	...	" C	90	120	78	65
39	" "	...	" D	100	72	72	76

From the data supplied in this table the following inferences can be drawn :—

1. A concentration of 1 : 12,800,000 of reduced tryparsamide thioglycollate suffices to kill the normal strain of trypanosomes within a period of four hours, provided the parasites are present in only moderate numbers of about 100 per 256 squares of the haemocytometer scale (*vide* Tube 21).

2. Such a concentration of drug is, however, insufficient to damage the trypanosomes of either strain, within a period of one hour, when the parasites are present in such enormous concentration as 15,000 per 256 squares of the haemocytometer scale : this is proved by the fact that the trypanosomes, which had been in contact with the drug for the initial hour, and had then been washed and suspended in moderate numbers in fresh nutrient medium, survived as well as the controls which had not previously been in contact with the drug (compare Tubes 36 and 38 with Tubes 37 and 39).

3. Although the suspension of very numerous trypanosomes for one hour at 37° C. in a concentration of 1 : 12,800,000 of reduced tryparsamide fails to damage the parasites, the medium in which the normal trypanosomes were incubated has at the end of the hour lost all its reduced tryparsamide : this is proved by the fact that it is no longer capable of destroying small numbers of the normal strain when they are subsequently incubated in it for many hours (compare Tubes 1-4 with Tubes 15-20). The death of the parasites in Tube 1 after incubation for twenty-four hours is not to be attributed to the presence of drug in the medium : the same phenomenon is observed in Tube 5 which contains no drug. In these cases the death of the trypanosomes is due to the removal from the nutrient medium of some essential constituent other than sugar (probably of a protein nature) resulting from the previous incubation in it of large numbers of trypanosomes for a period of one hour. This phenomenon is more fully discussed in one of our previous papers (1929).

4. In striking contrast to the behaviour of the normal strain the resistant parasites failed to remove any appreciable quantity of reduced tryparsamide from the nutrient medium. When this was subsequently examined by the biological method, almost as much drug was detected as was present in the nutrient medium, to which

drug alone and no trypanosomes had been added (compare Tubes 7-12 and Tubes 15-20).

5. The mere incubation of reduced tryparsamide in the nutrient medium for one hour before the addition of the trypanosomes is sufficient to reduce slightly the trypanocidal action of the drug (compare Tubes 15-20 and Tubes 21-26). Reference has been made to this fact in a previous paper (1930).

A number of experiments of this kind has enabled us to reach the general conclusion that the normal trypanosomes are capable of absorbing reduced tryparsamide much more readily than are the resistant trypanosomes. The latter strain will not absorb the drug, even if it is present in such concentrations as are rapidly fatal to the normal strain.

From observations of this nature it appears reasonable to infer that the resistance to the aromatic compounds of arsenic enjoyed by the atoxyl- and acriflavine-resistant strains is due to a relative impermeability of such trypanosomes to these drugs.

Whilst this fact is in harmony with Ehrlich's hypothesis of arsenic-resistance, or with any hypothesis which postulates that drug-resistance is due to an impermeability of the parasites, it is difficult to see how it can be reconciled with the hypothesis of Voegtlin, Dyer and Miller that a trypanosome strain owes its arsenic-resistance to a sufficient excess (above the physiological requirement) of sulphydryl compounds available for combination with arsenic. In short, we believe that the results of our experiments suffice to render Voegtlin's hypothesis untenable.

SUMMARY

1. Experiments are described which show that when normal and atoxyl-resistant strains of trypanosomes are incubated at 37°C. for one hour, in nutrient media, containing similar concentrations of reduced tryparsamide, their behaviour is essentially different, in that whilst the normal strain rapidly absorbs the drug, the atoxyl- (or acriflavine-) resistant strain fails to remove any appreciable quantity of the drug from the medium.

2. This fact is in harmony with Erlich's theory that drug-resistance is due to a lowered avidity of the specific chemoreceptors

of the parasite, and also with any hypothesis which explains drug-resistance on the basis of an increased impermeability of the parasite for the drug. It appears to render untenable the hypothesis of Voegtlin, Dyer and Miller, which postulates that a trypanosome strain owes its arsenic resistance to a sufficient excess (above the physiological requirement) of sulphydryl compounds available for combination with arsenic.

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THE DEMONSTRATION OF VIABLE LEISHMANIA IN THE FAECES OF EXPERIMENTALLY INFECTED BED-BUGS

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Although no work has, in recent years, been done in order to test the possibility of bed-bugs acting in nature as vectors of the Leishmaniases, very considerable numbers of investigations have, in the past, been directed towards this object.

It was shown by Patton (1907) that if bed-bugs (*Cimex hemiptera*) are fed on the peripheral blood of certain cases of kala-azar, the parasites assume the flagellate form and multiplication takes place within the intestinal tract of the bugs. Wenyon (1911) and Patton (1912) showed that a similar development takes place in the intestinal canals of bed-bugs which have fed over an unbroken oriental sore, or in the neighbourhood of a sore. In order conveniently to study the fate of Leishmania species once established within the bed-bugs, the device was adopted by Cornwall and La Frenais (1916) of feeding bugs artificially on a fluid consisting of a mixture of citrated rabbits' blood, and the water of condensation from cultures of *L. donovani* in N.N.N. medium, and it was shown that flagellates could subsequently be found in the intestines of the bugs for a period extending over some weeks. Patton and his co-workers (1921) demonstrated that by sterilising the outer surfaces of bugs infected in this way, dissecting out their intestinal canals and culturing the latter, together with their contents, in N.N.N. medium, successful growths of Leishmania could be obtained after the following periods since the infecting feeds :—44 days in the case of *L. tropica*, and 41 days in the case of *L. donovani*.

However, much experimental work, by many investigators, has completely failed to suggest any probability of Leishmaniasis being transmitted by the bed-bug to man or any susceptible animal by the anterior route, during the act of biting. Very slight attention has been paid to the possibility of viable Leishmania occurring in the dejecta of infected bugs, and such forms have not, up till now, been demonstrated. In the opinion of Patton (1921) the transmission of kala-azar in nature is effected by crushing infected bugs on the skin.

The possibility of the faeces of infected bed-bugs acting as infective agents in kala-azar did receive attention from Shortt and Swaminath (1925) who published a paper, at the beginning of which they announced that the discovery by Patton of the development of Leishmania into the flagellate form in the bed-bug, . . . ' instead of advancing our knowledge of the means of transmission of the disease, has acted rather as a deterrent to the careful investigation of other possible insect carriers.' Admitting that it had been . . . ' with the intention, therefore, of removing this obstacle,' they carried out experiments in order . . . ' to settle finally whether infection could occur either directly or indirectly, through the bite of the bed-bug *Cimex hemiptera*,' or by the ingestion of the bugs' faeces by an experimental animal. The animal used was *Macacus rhesus*, which they state had, in their experience, proved susceptible to the inoculation of splenic or liver emulsions in over 78 per cent. of cases. Twenty-five of these animals were subjected to the bites of a large number of bed-bugs which had fed on untreated cases of kala-azar. At varying intervals of time many of these bugs were again fed alternately on cases of kala-azar and the monkeys. After feeding on the monkeys, the shaved areas to which the bugs had been applied were rubbed thoroughly with a glass rod, in order, if possible, to produce infection of the bite-wounds with any faeces passed by the bugs during feeding. The duration of these experiments was over a year, but no infections resulted. In order further to test the possible infectivity of the faeces of the bugs used in these experiments, these were passed on to folded filter paper within the tubes in which the bugs were kept. The papers were later pounded up in a mortar with normal saline solution and fed to a monkey (only one animal being used for

this particular purpose). In this case, also, no infection resulted. One criticism of these experiments is that no attempt had apparently been made to determine whether any of the bugs used had become infected when they were fed on the cases of kala-azar. It had been insisted on by Patton (1921) that it is not all cases of kala-azar which can infect bed-bugs and it is only those cases in which there are large numbers of parasites in the circulating blood that can do so. Further, in regard to the experiment in which a monkey was fed on the faeces of bed-bugs, this cannot be regarded as conclusive, even assuming that some of the bed-bugs used were infected. No mention is made, in the account of the experiment, of the faeces having been administered soon after being passed by the bug; the faeces were apparently allowed to become desiccated, and under the conditions of the experiment one would only have expected a positive result if some resistant form of Leishmania were present in the bugs' excreta, and there is, as yet, no evidence of any such form.

That the parasites are still infective to experimental animals after a sojourn within the bed-bug has been shown by Mackie, Das Gupta, and Swaminath (1923) who infected a mouse by injecting intraperitoneally the intestinal contents, emulsified in saline, of seven bugs which had fed seventeen days previously on spleen-juice derived from active cases of kala-azar. Shortt and Swaminath (1923) similarly infected a mouse with the dissected guts, emulsified in normal saline, of twenty bugs fed nine days previously on the peripheral blood of cases of kala-azar.

The object of the experiments described below has been to demonstrate that viable forms of Leishmania can be passed in the faeces of infected bugs (*Cimex lectularius*).

I. STRAINS OF PARASITE EMPLOYED

Experiments were conducted with the following strains:—

(i) *Leishmania tropica:* From a human oriental sore, which had been produced experimentally from a canine strain originating in Baghdad.

(ii) *L. donovani:* * From an Indian case of kala-azar.

(iii) *L. donovani** var. *infantum:* Originating in Greece.

(iv) *L. braziliensis:* A laboratory strain brought back from South America by Professor Warrington Yorke.

(v) In addition to the above strains of Leishmania, it was thought advisable to repeat the procedures described below with a purely insect flagellate. The strain used was one of *Herpetomonas culicidarum*,* from *Culex pipiens*.

II. METHOD OF FEEDING BUGS ON INFECTED FLUID

A. *The feed.*

In the earlier series of bugs (Nos. 1-9 in the table below) used in these experiments, the feed consisted of a mixture of defibrinated rabbit's blood and flagellates from cultures maintained on the medium employed by Adler and Theodor (1926). Later, however, a culture medium prepared in the following way was used :—

Defibrinated rabbit's blood, 1 volume, is laked by shaking up with distilled water, 4 volumes. To this laked blood is then added 0·2 per cent. glucose solution in distilled water, and 6·3 per cent. NaCl solution in distilled water, in the following proportions—laked blood : glucose solution : salt solution :: 5 : 2 : 1. The medium is then distributed among test-tubes, 2 to 5 c.c. in each tube. Such a medium has the advantage, from the point of view of the experiments to be described, that it contains no agar, and bugs would readily feed directly on the cultures, without any addition of fresh blood. The growth occurs as a considerable deposit at the bottom of the tube, and it was from the lower part of the culture tubes that the feeding fluid was obtained. All the bugs from No. 10 onwards, shown in the table below, were fed on material obtained in this way.

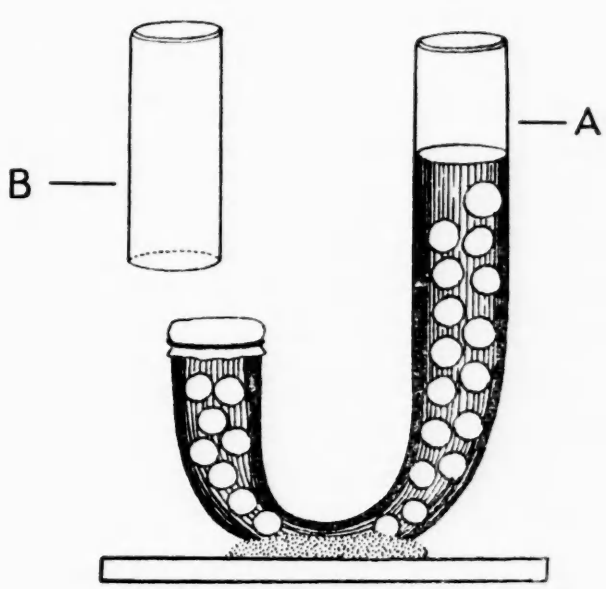
B. *Feeding apparatus.*

The apparatus used for feeding bugs consists of a piece of glass tubing, the diameter at its ends being about 1 cm. ; the tube has a **U**-bend, and one arm of the **U** is shorter than the other, as shown in the diagram (A).

The tube (A) is filled with small glass beads so as to minimise the amount of feeding fluid that will be required. After sterilisation the tube is fixed on a support, by means of plasticene, so that its arms point vertically upwards. The feeding fluid is then run into

* Kindly given by Professor J. G. Thomson, London School of Hygiene and Tropical Medicine.

the tube until the upper level of the shorter arm is reached, when the latter is covered by a mouse skin, so that the surface of the skin is uppermost; the skin is removed under clean conditions, but without particular regard to antisepsis, from the shaved abdomen of a recently killed mouse. Once the mouse skin has been fastened to the shorter limb of the tube by an elastic band, a little more fluid is introduced into the longer limb. The apparatus is now ready for use.



Apparatus used for feeding bed-bugs.

Starved bugs are allowed to crawl on the mouse skin, and, as a rule, will be found to feed within a few minutes. It was found useful to rest a short straight piece of glass tubing (B) on the upper surface of the shorter limb of the tube, to prevent the bugs from falling over the edge in wandering about on the mouse skin. In some cases where bugs appeared disinclined to feed, it was found an advantage to place the whole apparatus, together with the fluid, in the incubator, at 37° C., for about ten minutes before again offering the meal.

III. METHODS OF EXAMINING FAECES OF INFECTED BUGS

In order to collect faeces from infected bugs, the latter were allowed to feed on a human subject, at varying intervals after the primary feed on infective material. In most cases the bug would, within a minute or two after a full meal, deposit a small amount of

black, fluid faeces near the point where it had fed. On several occasions faeces were passed even before the bug had settled down to feed, soon after coming in contact with the warm skin.

The object in view being to demonstrate the presence of living forms of the parasite in the bugs' faeces, this was arrived at in most cases by sowing the latter, in the freshly passed condition, on the medium of Adler and Theodor. In order to avoid contamination of the cultures by organisms from the skin of the human subject, it was found sufficient to wipe the skin several times, first with absolute alcohol, and then with ether, before applying bugs to feed and defaecate on the arm. As soon as the excreta had been passed on to the arm of the subject, it was gathered up by means of a sterile glass pipette and planted on the medium. The great majority of cultures of bugs' faeces made in this way remained free from contamination by extraneous organisms. In some cases, in the later series of bugs employed, the excreta was examined microscopically in the fresh condition, immediately after being passed ; the bug was fed, and then placed on a glass slide ; as soon as it had defaecated on the slide a coverslip was applied, and the preparation examined.

Both sexes of bug were employed, and these in about equal numbers, and also both nymphal and adult forms, but these factors did not appear, in any way, to influence the course of events.

IV. RESULTS

The results are set out in the accompanying table (pp. 366-367).

It will be seen that successful cultures of *Leishmania tropica* have been obtained from the faeces of infected bugs from two to thirty-five days after their feed on the infective material.

In the cases where parasites were found on direct examination of the bugs' faeces, they were, in all instances, seen as active and vigorous flagellate forms, indistinguishable from forms met with in cultures on artificial medium. Dividing forms were encountered, and in some instances as many as six to ten flagellates were seen in every field of the microscope (objective 1/6). It was observed that although there may be many flagellates in the black faeces passed by the bug soon after its feed, these were not found in the clear fluid which follows the expulsion of the faeces.

Reference to the table below will show that several feeds on normal human blood subsequent to the bug's becoming infected do not seem to prejudice the appearance of parasites in its faeces at a later date.

V. CONCLUSION

Viable forms of Leishmania can be passed up to thirty-five days in the faeces of artificially infected bed-bugs, *Cimex lectularius*. The strains of Leishmania employed have been one each of the following :—*L. tropica, L. donovani, L. donovani* var. *infantum* and *L. braziliensis*.

Viable forms of a strain of *Herpetomonas culicidarum* have also been recovered from the faeces of artificially infected bed-bugs.

Table. To show the presence of parasites in the faeces of

Bug No.	Strain used.	Number of Days																						
		2	3	4	5	6	7	8	9	10	11	12	13	14	15	17	18	19	20	21	22	26	27	29
1	*L. tropica*	...	...	...	...	...	...	...	...	...	...	+	...	*f*	...	...	...	+	...	...	...	...	...	...
2		...	...	...	...	...	...	...	...	...	...	+	...	...	...	...	...	*f*	...	...	...	...	...	...
3		...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	*f*	+	K+	...
4		...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	+	...	...	...	...	...	...
5		+	...	...	...	...	...	...	*f*	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
6		...	...	...	...	...	...	...	...	...	...	...	+	...	...	...	...	...	...	...	+	...	...	...
7		...	...	...	...	...	+	...	...	...	...	...	...	...	...	...	...	...	−	...	...	...	...	...
8		...	...	...	...	...	...	+	...	...	...	...	...	...	...	...	...	...	...	*f*	...	...	...	...
9		...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	+	...	...	...	+	...	...	...
10		...	...	...	...	...	...	...	...	+	...	...	...	...	...	...	...	...	...	...	...	...	...	...
11		...	*f*	...	...	...	...	...	...	...	...	...	...	...	...	+*d*	...	...	...	...	...	...	...	...
12		...	...	...	...	...	...	...	...	+	...	...	...	...	...	...	...	...	...	...	...	...	...	...
13		...	...	...	...	...	...	...	...	...	+*d*	...	...	...	...	...	...	...	...	...	...	...	...	...
14		...	+	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	−
15	*L. donovani*	...	...	...	...	...	+*d*	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
16		...	...	...	...	...	...	+*d*	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
17		...	...	...	...	+*d*	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
18		...	...	...	+*d*	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
19	*L. infantum*	...	...	...	...	...	...	+*d*	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
20		...	...	...	+*d*	...	...	...	..	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
21		...	...	...	...	...	+*d*	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
22	*L. braziliensis*	...	...	+*d*	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
23		...	...	...	+*d*	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
24		...	...	...	+*d*	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
25		...	...	...	...	...	...	...	...	+	...	...	...	...	...	...	...	...	...	...	...	...	...	...
26	*H. culicidarum*	..	...	...	..	...	...	...	...	...	...	...	...	...	+*d*	...	.....	..	...	...	...	...	...	...
27		...	...	...	...	...	...	...	...	...	...	...	...	...	...	+*d*	...	...	...	...	...	...	...	...
28		...	...	...	...	...	...	...	...	...	...	...	...	...	...	+*d*	...	...	...	...	...	...	...	...
29		...	...	...	+	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...

Explanation of Signs

\+ indicates bug has fed on human subject and defaecated. Successful culture of parasites from faeces.
+*d* indicates bug has fed on human subject and defaecated. Active flagellates seen on direct examination of faeces.
− indicates bug has fed on human subject and defaecated. No growth after planting faeces on culture medium.
f indicates bug has fed on human subject. Either no faeces passed, or culture medium contaminated after planting faeces.

bed-bugs at varying intervals after their infecting feeds.

AFTER INFECTING FEED.

31	32	33	35	39	40	42	44	45	46	47	48	50	52	53	55	57	62	63	64	66	67	83	103	124	128	132	149
...	...	...	...	...	*f*	...	*f*	...	...	...	–	...	...	...	...	...	–	...	...	...	...	*f*	...	D–	...	...	...
...	...	...	...	...	*f*	...	...	...	...	...	...	...	...	...	...	...	–	...	...	...	...	...	...	...	...	*f*	K –
...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
...	...	...	...	–	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	*f*	...	K–	...	...
...	...	...	...	...	...	D	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
...	...	...	...	...	...	–	...	...	...	...	...	...	...	...	...	–	...	...	...	–	K–	...	...	...	...	...	...
...	...	...	...	–	...	...	...	...	...	...	...	...	...	–	...	...	...	–	K–	...	...	...	...	...	...	...	...
...	...	...	...	–	...	...	...	...	...	...	...	...	...	...	...	...	...	–	K–	...	...	...	...	...	...	...	...
...	...	...	...	–	...	...	...	...	...	...	...	...	...	...	*f*	...	...	–	K–	...	...	...	...	...	...	...	...
–	...	...	...	...	...	...	...	...	...	–	...	...	K–	...	...	...	...	...	...	...	...	...	...	...	...	...	...
...	...	...	+	...	...	...	...	...	...	...	–	...	K–	...	...	...	...	...	...	...	...	...	...	...	...	...	...
...	...	+	...	...	...	...	...	...	–	...	...	...	K–	...	...	...	...	...	...	...	...	...	...	...	...	...	...
...	–	...	...	...	...	...	...	–	...	...	...	...	K	...	...	...	...	...	...	...	...	...	...	...	...	...	...
...	...	...	...	...	*f*	...	...	...	...	...	...	–	K	...	...	...	...	...	...	...	...	...	...	...	...	...	...
...	...	...	...	...	*f*	...	...	...	...	...	...	–	K	...	...	...	...	...	...	...	...	...	...	...	...	...	...
...	...	...	...	...	...	...	...	...	...	...	...	...	...

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THE NAMES OF SOME MOLLUSCAN HOSTS OF THE SCHISTOSOMES PARASITIC IN MAN

BY

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The object of this note is to draw attention to certain points in the nomenclature of some of the better-known intermediate hosts of the three species of *Schistosoma* parasitic in man. Great confusion prevails in medical, veterinary and helminthological literature in the names used for these hosts, and the names in current use are frequently not in accordance with the international rules of zoological nomenclature. In putting together these observations I have had the advantage of the kind help and guidance of Major M. Connolly and of Mr. G. C. Robson, to both of whom I am very greatly indebted. Thanks are also due to Mr. J. R. le B. Tomlin for his valuable help in consulting some of the literature.

The known or suspected snail hosts of the human Schistosomes almost all fall into four genera : *Bulinus*, *Physopsis*, *Planorbis* and *Oncomelania*. These may be usefully discussed in order.

Bulinus [Adanson, 1757, pre-Linnean] Müller, 1781. The correct spelling of this name is *Bulinus*, not *Bullinus* (which was a variant introduced by Oken in 1815), and not *Bulimus* Scopoli, 1777 (a doubtful genus belonging, in any case, to a different family). For a discussion of the status of *Bulinus* the reader may be referred to Pilsbry and Bequaert (1927), pp. 132-135. According to these authorities *Isidora* Ehrenberg, 1831 (among other names) falls into the synonymy of *Bulinus*. It is frequently treated as a subgenus of *Bulinus*, but this is apparently incorrect, and the name *Isidora* should disappear entirely from the literature.

With regard to the nomenclature of the species of *Bulinus*, the three names in most general use in medical and helminthological literature for the forms of *Bulinus* acting as intermediate hosts for

Schistosoma haematobium in Egypt and Northern Africa are *B. contortus* (Michaud), *B. innesi* Bourguignat, of Pallary, and *B. dybowskii* Fischer, of Pallary. The last is commonly misspelt *dybowski* or *dybowskyi*. An important paper by Annandale (1922) appears to have been almost entirely overlooked by those who have written about these forms. Annandale examined examples of *Bulinus* from a large area, including the South of France, Portugal, Corsica, Algeria, Egypt and Mesopotamia. In several of these localities he studied living examples in their natural habitat. As the result of his work he states his opinion that " all the Bullininae of Western Asia and North Africa (except perhaps *B. raymondianus*) belong to one species. *Physa contorta* Michaud (1831) becomes, therefore, a synonym of *P. truncata* (Fér.) Audouin, the original figure of which represents a young shell of the form common in Lower Egypt." The various local strains of the North African form of *Bulinus*, and their different growth-stages, grade into each other in such a way that it is, in fact, as Major Connolly has shown me, impossible to separate them as distinct species. The conclusion is inevitable that the synonymy given by Annandale must be accepted. This includes *contortus*, *dybowskii* and *innesi*. The name *B. truncatus* seldom, if ever, appears in current literature, but apparently should replace all the others mentioned. *Bulinus tropicus* (Krauss) is a distinct South African form which has been implicated as a host of *Schistosoma mansoni*.

Physopsis Krauss, 1848, was originally proposed as a monotypic genus, with the species *africana* Krauss. This genus calls for no special comment. *P. africana* is commonly mentioned in connection with *Schistosoma haematobium* in South Africa, where, according to some authorities, it also harbours *S. mansoni*. *P. globosa* (Morelet), which has been incriminated as a host of *S. haematobium* in West Africa and in Nyasaland, is sometimes treated as a sub-species of *P. africana*, but is regarded by Major Connolly as possibly a distinct species.

Planorbis Müller, 1774, has been divided by conchologists into a number of sub-genera. *Planorbis boissyi* Potiez and Michaud, and *P. pfeifferi* Krauss, according to Major Connolly, should be referred to the sub-genus *Planorbula* Haldeman, 1840, since both, when young, possess the " teeth " inside the mouth of the shell which are

characteristic of that sub-genus. These teeth are a very rare feature in *P. pfeifferi*, but have been observed by Major Connolly in examples from several localities. These two African hosts of *S. mansoni*, therefore, which are referred by Germain and Neveu-Lemaire (1926) to the sub-genus *Planorbis*, should be called *P.* (*Planorbula*) *boissyi* and *P.* (*Planorbula*) *pfeifferi*.

The correct name of the form which serves as a host for *S. haematobium* in Portugal, and is variously referred to in the literature as *P. corneus* var. *metidjensis* or as *P. metidjensis* var. *dufouri*, is *Planorbis* (*Planorbis*) *dufourii* Graells. This is the name applicable to the Iberian form. *P. metidgensis* [*sic*] Forbes is the name applied to the closely related Algerian form.

The New-World species, *Planorbis olivaceus* Spix, *P. guadaloupensis* Sowerby (often misspelt "*guadeloupensis*"), *P. centimetralis* Lutz and *P. antiguensis* Sowerby,* which serve as hosts for *S. mansoni*, are referable to the sub-genus *Planorbina* Haldeman, 1843.

Oncomelania Gredler, 1881. All the known intermediate hosts of *Schistosoma japonicum* are now referred to this genus. Annandale (1924) divided the species of *Oncomelania* into two "sections," and in this he is followed by Germain and Neveu-Lemaire (1926), who define these sections as the sub-genera *Hypsobia* Heude, "1882" and *Hemibia* Heude, "1889." † *Katayama* Robson, 1915, falls as a synonym of *Hypsobia*. *Blanfordia* Adams, 1863, has been used as the generic name for *O. nosophora* and other species of *Oncomelania*, e.g. by Pilsbry,‡ but according to Annandale (1924) and Thiele (1929), this is a genus distinct from *Oncomelania*.

Oncomelania nosophora (Robson, 1915) and *O. formosana* (Pilsbry and Hirase, 1905) fall into the sub-genus *Hypsobia*, while *O. hupensis* Gredler, 1881, falls into the sub-genus *Hemibia*. *Katayama fausti* Bartsch, 1925, is, in Mr. Robson's opinion, possibly a form of *O. nosophora*. In any case it belongs to the sub-genus *Hypsobia*.

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* This name seems to have been first published by Sowerby in Reeve's *Conchologia Iconica*, Vol. XX, *Planorbis*, Pl. XI, species 92. The date of publication of this plate is uncertain, but from its appearance in the *Zoological Record* for 1877, it seems probable that it was published in that year.

† Heude mentions *Hemibia* in 1889 (*Journ. de Conchyliol.* (3), XXIX, p. 47), but without definition. It appears to have been first defined by him in 1890 (*Mém. concernant l'Hist. nat. de l'Empire Chinois*, Chang-Hai, I, 4e. cahier, p. 168). The definition of *Hypsobia* quoted by Germain and Neveu-Lemaire was also given by Heude in the latter work (1890, not 1882), p. 173.

‡ *The Nautilus*, XXIX, p. 1 (1915).

The classification of these forms is still mainly based upon the characters of the shell. A full account of the anatomy of *O. nosophora* has been given by Robson (1921).

To sum up, while no attempt is made here to give a complete list of the vectors of the human Schistosomes, the following list of corrected names may serve to make clear the conclusions reached from the foregoing discussion.

Hosts of *Schistosoma haematobium.*

Bulinus truncatus (Audouin, 1826).
(Synonyms : *contortus, dybowskii, innesi,* etc.)
Physopsis africana Krauss, 1848.
Physopsis globosa (Morelet, 1866).
Planorbis (*Planorbis*) *dufourii* Graells, 1846.

Hosts of *S. mansoni.*

Planorbis (*Planorbula*) *boissyi* Potiez and Michaud, 1838.
Planorbis (*Planorbula*) *pfeifferi* Krauss, 1848.
Planorbis (*Planorbina*) *olivaceus* Spix, 1827.
Planorbis (*Planorbina*) *guadaloupensis* Sowerby, 1821.
Planorbis (*Planorbina*) *centimetralis* Lutz, 1918.
Planorbis (*Planorbina*) *antiguensis* Sowerby, in Reeve [1877 ?].
Physopsis africana Krauss, 1848.
Bulinus tropicus (Krauss, 1848).

Hosts of *S. japonicum.*

Oncomelania (*Hypsobia*) *nosophora* (Robson, 1915).
Oncomelania (*Hypsobia*) *formosana* (Pilsbry and Hirase, 1905).
Oncomelania (*Hemibia*) *hupensis* Gredler, 1881.

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A NOTE ON ALAPLECTANA NOV. GEN.* SUB-FAMILY COSMOCERCINAE RAILLIET, 1916 FAMILY OXYURIDAE COBBOLD, 1864

BY

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(*Received for publication* 17 *June*, 1931)

The material was kindly put at the writer's disposal by Professor Khalil Bey, to whom I wish to express my gratitude. It consisted of two nematodes, a male and a female, that were found in the small intestine of an amherst pheasant, *Crysolophus amherstae*, that arrived from China and died in Cairo Zoological Gardens in December, 1930, after a period of six months.

The males are slightly smaller than the females. Mouth surrounded by a pair of fleshy lips with a small papilla on the anterior extremity of each. There is a vestibule with complicated teeth at the base. The oesophagus is divided into a short anterior part and a long posterior part which are separated from each other by a thick muscular band. The cuticle is provided on each side with a broad lateral phalange.

MALE. Caudal extremity is ventrally curved. There are two equal spicules and a gubernaculum. There are two narrow caudal alae with ten pairs of papillae.

FEMALE. Oviparous. The vulva is placed at the junction of the middle and posterior third of the body.

Type species *Alaplectana amhersti*

* This name was suggested owing to the close relationship between the new genus and *Aplectana*.

ALAPLECTANA AMHERSTI nov. sp.*

MALE. The male is 10 mm. in length by 1 mm. in diameter. The female is 15 mm. in length by 1·5 mm. in diameter. The body of the male is curved ventrally towards the tail which tapers to a fine point. The female is practically straight, the tail gradually ending in a fine point. The cuticle expands laterally from the head towards the level of the posterior border of the oesophagus in the form of two broad lateral phalanges ; each is 55μ in breadth. The mouth opening is guarded by two fleshy lips, each with a papilla on the anterior end of the lip. There is a vestibule about 36μ in depth.

Pharynx. The anterior part of the oesophagus is a short canal, the walls of which are muscular and are separated from the posterior part by a thick muscular band. From its cephalic end arise a few complicated teeth directed anteriorly. Their shape and number could not be ascertained.

Oesophagus. The posterior part is ·72 mm. and ends in a bulb with a valvular apparatus. The bulb is ·22 mm. by ·15 mm.

Tail. The caudal alae are very narrow. There are ten pairs of caudal papillae. Eight pairs are pedunculated and two are sessile. The latter lie on each side of the anus. Six pairs of the papillae are post-anal and four are pre-anal.

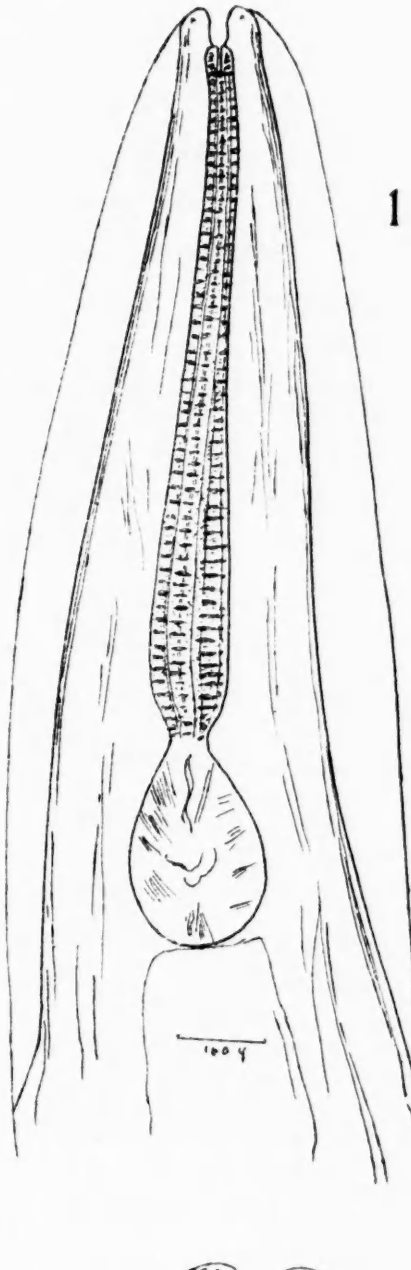
Spicules are equal. Each is 1·18 mm. in length. Gubernaculum is present and is 50μ in length by 15μ in breadth.

FEMALE. The genital tract is distended with eggs that make the details difficult to describe. There are two uterine tubes and the vulva opens at the junction of the middle and posterior thirds of the body.

DISCUSSION

The genus belongs to the family *Oxyuridae* in virtue of the oesophageal bulb, the double uterine tubes and the absence of a pre-anal musculature or sucker. The fact that there are two equal spicules and a gubernaculum places it in the sub-family *Cosmocercinae.*

* The specimens are kept in the Museum of the Parasitological Department of the Faculty of Medicine, Cairo.



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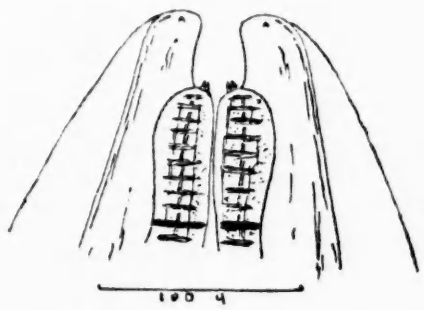

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Alaplectana nov. gen. 1.—Anterior part of the nematode with the phalanges and oesophagus; 2.—Vestibule and teeth; 3.—Caudal extremity of the male.

The only genera that are related to the present one in having narrow phalanges are :—

1. *Cosmocercella*, which is characterised by the presence of three lips, two caudal pouches in the male and two rows of plectanes.
2. *Aplectana* has three lips, almost indistinct lateral phalanges, and the members are parasites of amphibian reptiles.
3. *Syphaciella* has again three lips, very narrow phalanges, and is characterised by the presence of broad caudal alae and no pedunculate papillae.

Since it is the universal practice to use such characters as the number of lips, the presence of broad lateral phalanges and the nature of the host for defining genera, I propose the name *Alaplectana* for the new genus to contain the present species.

Modification of the Key to the genera given by Yorke and Maplestone.

Cosmocercinae Railliett, 1916

Oxyuridae, male, with two equal spicules, gubernaculum present.

1.	Posterior extremity of both sexes, blunt and round	*Amblyonema*
	Posterior extremity of both sexes are finely pointed	2
2.	Broad cervical alae	*Alaplectana* n. gen.
	Cervical alae very narrow or absent	3
3.	Males with plectanes	4
	Males without plectanes	5
4.	Males with a pouch-shaped caudalala on each side of cloaca	*Cosmocercella*
	Males without pouch-shaped caudal alae	*Cosmocerca*
5.	Males with wide caudal alae and about three caudal papillae	*Syphaciella*
	Males with caudal alae very narrow or absent and about twelve caudal papillae	*Aplectana*

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SUSCEPTIBILITY AND RESISTANCE TO TRYPANOSOME INFECTION

VIII. — *IN VITRO* DEMONSTRATION OF SPECIFIC AGGLUTINATING AND TRYPANOLYTIC ANTIBODIES IN THE SERUM OF INFECTED GUINEA-PIGS

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The course of a trypanosome infection in guinea-pigs, rabbits, and higher animals is characterised by its intermittent character. The mechanism of the relapsing nature of the infection has not as yet been adequately determined. The results of our previous investigations indicated that the mechanism of relapse as well as that of resistance to re-infection was associated with the cellular defences of the body. It was shown that on the one hand, relapse can be brought about by exhaustion of the cellular defence by injection of olive oil into guinea-pigs (Kligler and Weitzman, 1924), and that on the other, splenectomy greatly lowers the resistance of rats to an infection (Kligler, 1929).

Although these findings helped to explain, partially at least, the nature of the primary resistance to an infection and the mechanism of relapse, they did not account for the sudden critical disappearance of trypanosomes in infected animals, more particularly in the guinea-pig.

The recent work of Taliaferro and Johnson (1926), and Johnson (1929), as well as the earlier work of Massaglia (1907), Levaditi and Muttermilch (1909), Levaditi and McIntosh (1910), and Leger and Ringenbach (1911), showed that following a trypanolytic crisis in guinea-pigs, there is a specific lytic substance present in the serum.

Massaglia showed that the serum of an animal which was slightly lytic before a crisis, became strongly so after a crisis. Levaditi and Muttermilch confirmed these findings and added the observation that a strain of trypanosome could be rendered resistant to the serum *in vitro* by a few minutes' contact with it. Levaditi and McIntosh repeated and confirmed these experiments and were able to develop resistant strains by *in vitro* contact with immune serum. In these experiments most of the trypanosomes were killed but a few remained and these, when injected into mice, gave rise to strains which were resistant to the action of the particular immune serum. These authors interpreted the process as one of selection. Rosenthal (1913), however, postulates the presence in the serum of a substance, antagonistic to the lytic substance, which increases the resistance of trypanosomes to the action of serum.

Our own attempts to demonstrate humoral antibodies either *in vivo* or *in vitro* have given negative or variable results. Recently Yorke and his associates (1929, 1930) were able to demonstrate *in vitro* a pronounced trypanolytic effect of normal human serum on certain pathogenic trypanosomes non-infective for man. Although the peculiar trypanocidal property of human serum has been well known and the subject of numerous investigations, previous investigators have failed to observe this effect *in vitro*. According to these workers, it is essential that the number of trypanosomes inoculated into the testing media be small, or else the results are obscured. These studies have prompted a re-investigation of the trypanolytic properties of guinea-pig and rat serum following a crisis, i.e., following the sudden disappearance of trypanosomes from the circulation.

EXPERIMENTAL

In these experiments the technique differed in some respects from that employed by Yorke and his associates. The testing medium was a diluted serum, consisting of 5 parts guinea-pig serum, 3 parts glycosal solution and 2 parts phosphate buffer mixture, M/30 of PH 7·8. The tubes were incubated at 25° C. to 28° C. Under these conditions the trypanosomes remained viable and infectious for at least three days and the effect of the serum could be followed without any difficulty.

The usual procedure was as follows : 1·0 c.c. of the serum-glycosal mixture was added to a series of tubes. To these were added, first, decreasing dilutions of the serum to the tested, and then equal amounts of a trypanosome suspension taken from an infected rat. The amount of trypanosome suspensions added was usually graded to give a final concentration of not more than 5,000 trypanosomes per cubic millimetre of medium. The suspension was then placed in the incubator and examined at stated intervals. In some experiments rats were infected after given periods in order to ascertain the infectivity of the treated trypanosomes.

Each experiment reported below has been repeated two or three times, but it will suffice to present here protocols of typical experiments.

Experiment 1. To the serum-glycosal solution were added different dilutions of a serum taken from a guinea-pig after the first 'crisis'. Each tube contained 700 trypanosomes per cubic millimetre and the tubes were incubated at 28° C. and examined at various intervals under the dark field microscope. The results are tabulated below :—

Time interval	Serum Dilutions.						Control
	5	10	20	50	100	500	
2 hours	All dead, bizarre forms, swollen.	All dead, swollen and granulated.	Rare motile form ; mostly dead and swollen.	Viable ; actively motile ; adhesion ; few clumps.	Active ; no dead forms.	Actively motile.	Actively motile.
24 hours	All dead ; largely dis-integrated.	All dead ; largely dis-integrated.	Mostly dead ; rare live forms, sluggishly motile.	Many clumps ; few live forms ; still active.	Active ; no dead forms.	Actively motile.	Actively motile.
2 days	All dead ; largely dis-integrated.	All dead ; largely dis-integrated.	All dead and dis-integrated.	Few live active trypano-somes.	Active ; no dead forms.	Actively motile.	Actively motile.

This experiment was repeated, using defibrinated blood in place of serum. The results were the same. Active lysis occurred during the first two hours in serum dilutions up to 1 : 20. Longer time intervals did not materially change the results. Serum dilutions of 100 and 500 had no effect at all, whereas a dilution of 1 : 50 exerted agglutinating and only partially lytic action.

EXPERIMENT 2. The purpose of this experiment was to ascertain whether complement played a part in the lytic process. The substrate was the same as above except that in one set of tubes the guinea-pig serum was inactivated by heating at 55° C. for thirty minutes. The immune serum was added in dilutions 10 and 20. The trypanosomes were from an infected rat, the final concentration being 2,000 per mm^3. The tubes were incubated at 28° C. and examined at various intervals.

Interval	Unheated Serum		Heated Serum		Control
	1 : 10	1 : 20	1 : 10	1 : 20	
2 hours	Many clumped swollen masses ; few free motile forms sensitive to light.	Same as in dil. 1 : 10	Agglomerated clumps ; flagellar ends motile ; few free and motile.		Actively motile.
24 hours	Complete lysis.	Complete lysis.	Occasional live trypanosome; sluggishly motile.		Actively motile.

The experiment was repeated with both rat and mice passage strains with the same results. In the tubes containing unheated serum lysis was more prompt and agglomeration less in evidence than in the tubes containing inactivated serum. The ultimate effect was the same because the agglomerated trypanosomes promptly disintegrated ; the true lytic process, however, appears to depend on the presence of complement. In this respect, the behaviour of these antibodies is similar to bacterial antibodies ; agglutination occurs in the absence of complement but lysis requires the presence of complement.

EXPERIMENT 3. The object of these experiments was to determine the behaviour of the serum of an infected animal after a

second pyrexial crisis. The serum was drawn at the end of the first relapse and tested against the regular passage strain as well as against the relapse strain started from this guinea-pig in rats.

The results of the first experiments against the passage strain showed that the serum was still actively lytic in dilutions of 10 and 20 respectively, in one to two hours. Further tests revealed, however, the fact that it was inactive against the relapse strain and, moreover, that strains could be selected *in vitro* which in the early passages in rats examined, remained resistant to the effect of the serum.

A. In this experiment a guinea-pig serum taken after the second pyrexial crisis was tested at the same time against the passage (*p*) and relapse (*r*) strain of trypanosome. Each series was set up with normal and inactivated guinea-pig serum respectively. The results are tabulated below :—

Strain	Serum	Immune serum dilution	Time Interval	
			1 : 30 minutes	24 hours
p	Normal ...	10	Complete lysis ; few agglomerated clumps.	No live forms seen.
	Normal ...	20	Complete lysis ; few agglomerated clumps.	No live forms seen.
	Normal ...	*C*	All active.	Actively motile.
p	Inactivated	10	Active clumps and free forms ; adhesion.	Reduction in numbers but still active.
		20		
		C	All free active.	Actively motile.
r	Normal ...	10	No lysis ; adhesion +	Reduction in numbers but still active.
		20	No lysis ; adhesion +	
		C	All active.	Actively motile.
r	Inactivated	10	No lysis ; adhesion +	Reduction in number but still active.
		20	No lysis ; adhesion +	
		C	All active.	Actively motile.

It is clear that this serum exerts only a mild effect on the relapse strain, whereas the passage strain was completely lysed in a dilution of 1 : 20 in one and a half hours. Incidentally, this experiment again illustrates the relation of complement to the lytic process.

B. This experiment was repeated and at the same time another serum taken after the first crisis was tested as a control. The two sera gave the same results. The passage strain was almost completely destroyed by a 1 : 20 dilution of the sera in one hour, while the relapse strain was affected only slightly.

After twenty-four hours 0·5 c.c. of the material from the respective 1 : 20 dilutions of the two sera was inoculated into rats. These rats were designated as *a* and *b* respectively; rat *a* received the trypanosome material treated with the relapse serum, and rat *b* that acted upon by the serum after the first crisis. After six days both rats developed an infection.

The effect of relapse serum (R) was now tested against strains *a*, *b*, as well as the relapse (*r*) strain. The results of this experiment are tabulated below :—

Trypanosome strain	Immune serum dilution	Number of trypanosomes after 2 hours/ c.mm.	Microscopic findings after 24 hours
a	10	2,300	Active forms ; some sluggish.
	Control	2,000	All active.
b	10	2,700	All active.
	Control	3,100	All active ; somewhat more numerous.
r	10	6,400	Equally active with the control.
	Control	5,500	

It is clear from this experiment that the immune serum did not destroy all the trypanosomes and that the few surviving forms, although too small in number to be detected by a cursory examination under the dark field microscope, were sufficiently virulent to infect rats. It is also apparent that the strains thus selected were resistant to the action of this serum after a rat passage, corresponding in this respect to the relapse strain in animals. Subsequent tests showed that this resistance is lost after many passages through the rat.

C. In this experiment, the action of three sera was tested on the regular passage strain. One serum was taken after the subsidence of the second relapse ; the second after the fourth relapse ; the third was a serum taken from a rat just prior to death.

Serum 1 produced partial lysis after one and twenty-four hours respectively in dilutions of 1 : 10 and 1 : 20.

Serum 2 gave partial agglutination in the 1 : 10 dilution but was entirely without effect in the 1 : 20 dilution.

Serum 3 (rat serum) had no effect at all.

It appeared, therefore, that in the course of the infection the

lytic substances present early in the disease tend to disappear. This experiment was repeated and counts made. The results are given below :—

Serum	Dilution	Count per c.mm.	After 1 hour	After 24 hours
			Microscopic picture	Microscopic picture
1 (second relapse)	10	2,500	Many dead clumps ; many still active.	Reduction in number but all free.
	20	3,600	Few clumps ; rest all actively motile.	
	C	5,600	All actively motile.	Actively motile.
2 (fourth relapse)	10	2,400	Partial agglomeration and lysis ; many actively motile.	
	20	2,600		
	C	2,600	No agglomeration or lysis.	Free ; actively motile.
3 (Rat)	10	8,200	All equally active ; occasional dead form.	Activity more sluggish than in 1 and 2. (This is due to the larger number of trypanosomes inoculated.)
	20	7,300		
	C	7,200		

It seems clear that in some animals at least there is a loss in lytic activity of the sera during the later stages of the infection as compared with those taken after the first crisis.

The results of these various experiments indicate that there are variations in the activity of sera of different animals ; that the sera do not affect each strain of trypanosome alike ; that it is possible to recover *in vitro* resistant strains which are no longer affected by the serum still active against the original passage strain and finally, that as relapses recur the lytic power of the serum against the passage strain tends to diminish.

Experiment 4. The variability in the lytic effect of sera is further illustrated by the following experiments. Guinea-pig B was infected with the passage strain. After the first pyrexial crisis the serum was tested against the passage and relapse strains respectively. The procedure was the same as in the previous experiments. The results are tabulated below. This serum was active against both strains but more markedly so against the relapse than the passage strain.

A. Immune serum B ; trypanosome strains *r* and *p* ; inoculum about 5,000 trypanosomes per cubic millimetre of fluid.

Strain	Dilution	After 2 hours		After 24 hours	
		Count per c.mm.	Microscopic findings	Count per c.mm.	Microscopic findings
r	10	90	Rare live form ; some dead clumps.	0	No live trypanosomes found.
	20	380	Many moving clumps and dead forms.	0	Few large dead clumps.
	C	2,180	All actively motile.	3,700	Actively motile.
p	10	40	Many dead ; some sluggishly motile.	0	No live forms seen.
	20	270	Motile clumps.	140	Occasional active forms
	C	1,400	All actively motile.	1,300	Very active.

B. Serum same as *A* ; *r* and *p* strains from new rats.

Strain	Serum dilution	After 1 hour		After 24 hours	
		Count per c.mm.	Microscopic findings	Count per c.mm.	Microscopic findings
r	10	20	Few dead clumps ; active lysis.	0	No live forms seen ; disintegrating clumps.
	20	10		0	
	40	1,600	Many free active ; few live clumps.	2,200	Many active ; few clumps.
	C	2,800	All free and active.	2,400	Actively motile.
p	10	4,700	Agglutination and lysis, but many still active.	2,100	Actively motile.
	20	2,800			
	C	11,800	Numerous free and active forms.	350	Only few live trypanosomes (due to heavy inoculum).

The relapse strain which had by this time passed through twenty-five passages in the rat, was more sensitive to this serum than the regular passage strain. In the subsequent experiments an attempt was made to ascertain the constancy of the relapse strain by testing

the cross lytic effect of sera of guinea-pigs infected with the passage and relapse strains respectively. Two sera were tested :—

Guinea-pig 2 infected with the relapse strain, and guinea-pig 8 infected with the passage strain.

A. Effect of both sera on the *relapse strain r.* Inoculum about 5,000 per c.mm.

Interval	Serum 8 (passage) dilution		Serum 2 (relapse) dilution		*C*
	10	20	10	20	
After 1 hour	Few still actively motile ; mostly dead forms.		Complete lysis.	Rare live forms.	Many actively motile.
24 hours	Few free forms ; many dead agglomerated clumps.		No live forms seen.	Two live trypanosomes in drop. Many dead agglomerated clumps.	Many actively motile.
48 hours	Few actively motile forms.		No live forms seen.	...	Many active forms.

B. Effect of both sera on the *passage strain p.*

Time interval	Serum 8 (passage) dilutions		Serum 2 (relapse) dilutions		*C*
	10	20	10	20	
After 1 hour	Few active forms ; mostly dead.		No live forms seen.		Many actively motile trypanosomes.

This experiment was repeated with sera from other guinea-pigs. As in the above experiment the sera no longer differentiated between the two strains, relapse sera being more active against the passage strain and vice-versa according to the general potency of the serum.

In general it was clear from the experiments that the potency of the sera varied with the animal just as the course of infection varies with different animals. This phenomenon is illustrated by the following experiment :—

Five guinea-pigs were infected at the same time, and at the end of the first crisis blood was taken and the serum tested against the same strains of trypanosome. The results are summarized below.

The guinea-pigs were infected 26/2. The course of infection was followed by daily examinations of the blood and when sufficiently numerous the number of trypanosomes per c.mm. recorded. Blood was taken at the first drop after a rise in the number of trypanosomes in the circulation.

Course of infection

1	2	3	4	5
No increase sufficient to count until 9/4, when number rose to 4,000, after that negative or occasional; typical skin and eye lesions. Bled 10/5.	9/3 positive 10/3 14,000 11/3 14,000 12/3 20,000 13/3 30,000 15/3 negative; bled	8/3 positive 9-15/3 negative to occasional 16/3 4,000 17/3 8,000 19/3 8,000 20/3 6,000 22/3 4,000 23/3 14,000 24/3 10,000 25/3 70,000 26/3 10,000 27/3 negative; bled	8-14/3 negative to occasional 15/3 20,000 16/3 28,000 17/3 6,000 17/3 6,000 19/3 20,000 22/3 4,000 23/3 4,000 24/3 6,000 25/3 10,000 26/3 negative; bled	16/3 8,000 17/3 8,000 19/3 2 per field 20/3 occasional; bled

Activity of Sera

Incomplete lysis in dilution 1:10, only partial lysis at 1 : 20 and none at 1 : 40.	Strain 1 Partial lysis in 1 hour and complete lysis 24 hours in 1 : 10 and 1 : 20. Strain 2 Complete lysis in 1 hour in dilutions 10 and 20.	Strain 1 Complete lysis in 1 hour in dilution 10 and 20. Strain 2 Partial lysis in 2 hours at 37° C. in dilution 20, but negative in 40 and 80.	Strain 1 Complete lysis in 1 hour at 28°C. in dilutions 10 and 20.	Strain 1 Incomplete lysis in 1 hour in dilutions 10 and 20.

EXPERIMENT 5. The object of this experiment was to ascertain the heat lability of the lytic antibody. A known lytic serum was divided into four portions, one of which served as control and the other three were heated in waterbaths at 60° C., 65° C. and 70° C., respectively, for thirty minutes. The potency of the different fractions was then tested against the rat passage strain. The results

of a typical experiment after incubation for twenty-four hours are given below.

Serum dilution	Unheated	Heated 60° C.	Heated 65° C.	Heated 70° C.	Control
1 : 10	Complete lysis.	Dead ; few dead clumps.	Complete lysis.	Many active forms.	Many active forms.
1 : 20	Complete lysis.	Dead clumps.	Active forms ; only slight effect.	Many active forms.	Many active forms.

It is apparent that the lytic antibody behaves like a bacteriolysin. It is partially destroyed by heating at 65° C. for thirty minutes and completely so at 70° C. Heating at 56° C. or 60° C. for thirty minutes resulted in no apparent diminution of the potency of the serum.

EXPERIMENT 6. This experiment was designed to ascertain whether the *in vitro* contact of trypanosomes with lytic sera completely destroyed the trypanosomes and rendered them non-infective for rats. Rats were infected with given quantities of trypanosome suspensions which had been in contact with lytic serum for varying lengths of time.

A. Sera from guinea-pigs 8 and 2 respectively were used. The same number (about 4,000 per mm.^{3}) of trypanosomes were inoculated into each tube, containing 1 to 10 and 1 to 20 dilutions of the immune sera. After contact for one hour, 0·5 c.c. of a given mixture was inoculated into a rat. All the rats developed an infection but the incubation period and duration of the illness varied with the different sera and dilutions used.

Serum	Dilution	Incubation days	Duration of infection days
8	10	2	6
	20	2	5
2	10	3	9
	20	3	7
Control	...	2	4

B. The same experiment was performed with the so-called relapse strain in place of the passage strain. The results are tabulated below :—

Serum	Dilution	Incubation days	Duration days	
8	10	...	...	Found eaten up; died from unknown cause.
	20	4	9	
2	10	6	12	
	20	5	9	
Control	...	2	5	

C. In this experiment sera 4 and 5 were used and the rats were inoculated with suspensions incubated for twenty-four hours. All the rats became infected, but in some cases the course was markedly prolonged as compared with the control :—

	Culture *r*				Culture *p*			
Serum	Dilution	Condition of trypanosomes	Incubation days	Duration days	Dilution	Condition of trypanosomes	Incubation days	Duration days
4	10	No live forms seen.	4	Eaten up, date unknown.	10	No live trypanosomes seen.	4	Eaten up, date of death unknown.
	20	No live forms seen.	5	13	20	No live trypanosomes seen.	5	11
5	10	All dead.	5	10	10	No live trypanosomes seen.	5	9
	20	Few live forms.	3	8	20	No live trypanosomes seen.	5	10
	C	Many active.	3	6	*C*	Many active.	4	9

D. This experiment was made with a mixture of sera taken from different animals. To a 1 to 10 dilution of the serum were added decreasing quantities of trypanosomes, the mixtures incubated

twenty-four hours at 28° C. and 0·2 c.c. inoculated into rats. A parallel test was made with media made up with inactivated guinea-pig serum. The results of this experiment were particularly interesting because none of the rats inoculated with the mixture containing normal guinea-pig serum became infected, while one of those receiving the inactivated serum mixture developed an infection after a prolonged period of incubation. The detailed protocol is given below :—

Number of trypanosomes per c.mm.	Active guinea-pig serum + Immune serum 1 : 10	Inactivated serum + Immune serum 1 : 10
		Incubation duration, days
4,000	No infection	No infection
2,000	No infection	10 22
1,000	No infection	No infection

In this experiment, therefore, using a mixture of several sera it was possible to obtain practically complete lysis so that the culture was no longer infective. Thus far this is the only experiment which has given such results. These results are, however, of interest from the standpoint of the polyvalent character of mixed sera and the experiments are being continued.

EXPERIMENT 7. The object of this experiment was to ascertain whether sera of rats taken at different stages of the infection contained demonstrable lytic antibodies. Tests were made *in vitro* with sera taken early in the infection, after the first crisis as well as during the middle and last stages of the infection. The technique was the same as that used in the case of guinea-pig sera. At no time was it possible to demonstrate the presence of lytic substances. The progressive destruction of trypanosomes in the rat (Kligler and Comaroff, 1929) is, therefore, essentially at least a cellular process. The following is the protocol of a typical experiment :—

Blood taken from three rats.

Rat 1 at the outset of the infection.

Rat 2 after a crisis.

Rat 3 just before death.

The history of rat 2 is of special interest : Infected 17/5, blood first positive 23/5, 24/5—8,000 per c.mm. ; 25/5—30,000 ; 26/5—48,000 ; 27/5—88,000 ; 28/5—20,000 ; 29/5 + in drop, but not numerous enough to count ; 30/5—blood taken for test ; 3/15—80,000 ; 6/1—90,000.

	Dilution	2 hours		24 hours	
		Count	Microscopical findings	Count	Microscopical findings
Rat 1 ...	1 : 2	3,600	All very active ; no clumps.	2,000	All equally active.
	2 : 4	2,700		1,550	
	C	4,200		1,200	
Rat 2 ...	1 : 5	1,390	Very active ; a few small active clumps.	940	All equally active.
	1 : 10	800	Very active.	530	
	C	860	Very active.	580	
Rat 3 ...	1 : 4	3,200	All active.	990	All active.
	C	4,200	All active.	1,090	All active.

Except for a mild agglutinating effect in rat 2, which had actually undergone a typical crisis, there was no suggestion of lysis either after two or twenty-four hours. These results correspond with those obtained previously in rats where the defence is apparently essentially cellular in character.

SUMMARY

A series of experiments are reported which confirm the findings of other investigators concerning the trypanolytic power of guinea-pig sera following a trypanolytic crisis. The lytic potency of these sera is relatively low ; some are still active in a dilution of 1 : 50, but more often the activity does not go beyond a dilution of 1 : 20. The results confirm the findings of Levaditi and Muttermilch concerning the rôle of complement in the lytic process and those of Levaditi and McIntosh and others regarding the selection of resistant strains by *in vitro* contact with immune sera. It was found, however, that after a series of passages in the rat these strains lose their resistance and revert in their susceptibility to the original passage strains. The heat resistance of the trypanolytic substance resembles that of bacteriolysins, being partially destroyed by heating at 65° C. and completely destroyed by heating at 70° C. for thirty minutes. The trypanolytic antibody in the serum of any one guinea-pig seems

incapable of destroying all trypanosomes in a suspension; even after contact of twenty-four hours, when no live forms can be found by an ordinary microscopic examination, the presence of pathogenic trypanosomes is still demonstrable by rat inoculation. In one instance only, when a polyvalent mixture of sera from different animals was used, was complete lysis obtained.

These *in vitro* experiments help to explain the course of events in the guinea-pig as well as the differences between the infection in the rat and that in the guinea-pig. It seems reasonable to assume that in the course of active development trypanosomes of different degrees of resistance exist. In the guinea-pig as in the rat multiplication and lysis proceed simultaneously, the number of trypanosomes in the circulation being a resultant of the relative rates of the two processes. It is conceivable that rapid multiplication results in the simultaneous development of a larger number of less resistant forms, as well as a higher concentration of specific lytic substances so that the balance between the two processes is disturbed and lysis is more rapid than multiplication. This balance between the two antagonistic processes accounts for the fact that in some guinea-pigs and in most rabbits the number of trypanosomes in the circulation is always very small. In these animals lytic substances are present even though there is no marked increase in the number of trypanosomes in the circulation; lysis presumably keeping pace with multiplication.

The following observation is illustrative. Guinea-pig 1 at no time developed any appreciable blood invasion. It lived two weeks longer than any of the other animals infected at the same time, and the serum of this animal taken three weeks after the infection was lytic in a dilution of 1 : 10, while the serum taken two months after the infection was still partially lytic against the passage strain in a dilution of 1 : 10 of the serum.

In rats no lytic substances could be detected either after a crisis or at any other stage of the infection. It is possible that these are present in such small concentration that they are not demonstrable *in vitro*. However, the effect of splenectomy on the infection and the mononuclear phagocytosis always observed in these animals point to the cellular defence as the principal one in this animal. The rate of destruction cannot keep pace with the rate of growth, and the parasites predominate so enormously as to destroy the host.

CONCLUSIONS

1. Serum of guinea-pigs infected with *Tr. evansi* contains trypanolytic substance, the concentration of which varies in different animals and in the different stages of the infection.

2. The lytic effect is specific, affecting only some of the trypanosomes in a suspension. Even twenty-four hour contact of serum and trypanosomes fails to destroy all of the trypanosomes in suspension. The remaining trypanosomes are still virulent and resistant to the action of the serum. This resistance is lost after repeated passage in rats, the strains becoming once more susceptible to the lytic effect of the serum specific for the passage strain.

3. In one experiment it was possible to obtain a mixed polyvalent serum which sterilized a suspension in twenty-four hours.

4. No corresponding trypanolytic substance could be detected in sera of infected rats.

5. As the infection progresses the lytic effect of the serum diminishes.

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SOME OBSERVATIONS ON THE STRUCTURE OF THE MOUTH PARTS AND FORE-INTESTINE OF THE FOURTH STAGE LARVA OF *AEDES (STEGOMYIA) FASCIATA* (FAB.)

BY

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Although it is true that several observers have described in some detail the mouth parts of the mosquito larvae, there are still many gaps in our knowledge of these structures. The present study was therefore undertaken to try and fill in some of these gaps. The larva of *Stegomyia fasciata* was selected mainly because an unlimited supply of eggs was available in the Department of Entomology. I wish to express my gratitude to Professor W. S. Patton for the invaluable assistance he has given me in innumerable ways. To him I owe the suggestion of this subject of investigation and the immense help I received during my work. I find it also impossible to express how greatly I am indebted to Dr. A. M. Evans, Lecturer at the School, for the keen interest she has taken in my work and for her unfailing kindness and the very ready and constant manner in which she has put at my disposal her great knowledge of mosquito larvae.

THE ANATOMY OF THE MOUTH PARTS

As is well known, the mouth parts of the mosquito larva consist anteriorly of the labrum-epipharynx, a median structure referred to in the literature as the palatum, which projects forwards between a pair of feeding brushes. On each side of the mouth opening there is a pair of maxillae, and dorsal to them a pair of large stout

mandibles. Behind the mouth opening is a complicated heavily chitinized plate referred to as the labial or mental plate which, at least in part, probably forms the labium or lower lip.

The labrum-epipharynx (Fig. I, No. 1; Fig. II, and Fig. III, No. 1). This structure has been referred to only in a casual manner by previous writers on this subject. Raschke (1887), in his work on the larva of *Culex nemorosus* (probably *Aedes punctor*), describes it as follows:—' Man kann drei Theile der Oberlippe unterscheiden, Zweiseitlich symmetrische und einen mittleren, den Linné und nach ihm Brullé palatum benennt.' Later Nuttall and Shipley (1901) described it in the larvae of *Anopheles*, writing: ' Between the bases of the brushes is a smaller bunch of hairs and ventrally there are two semi-circles of hairs, all above, in front of and converging on the mouth. The anterior median area which carries the brushes is called by Meinert the clypeus.' Imms later added: ' It seems probable that the sclerite should be regarded as clypeo-labrum.'

Thompson (1905), working on the larvae of *Culex* and probably *Aedes*, said: ' In the bay of thin chitin bounded in front by the anterior shelf and border line, and posteriorly by the black spot areas, lie the small palatum and the larger lateral flabellae.' The anterior shelf here refers to the clypeo-labral suture and the black spot areas to the base of the sclerite which articulates with the hair-bearing plate of the feeding brushes.

Howard, Dyar, and Knab (1912), describing it in the larva of *Culex*, wrote: ' Three parts of the labrum can be distinguished, two lateral portions and a median one which projects freely from the anterior margin of the head and has been called palatum.' Then follows an account of the way in which the mouth brushes act and a quotation from Thompson's paper. Later they again write: ' The palatum is clothed on its dorsal surface with short but coarse spine-like hairs, ventrally is inserted a long tuft of long pendant hairs which swing in unison with the lateral brushes. In the region of the epipharynx are two fringes of dense hairs arranged in closely approximated longitudinal series. According to Raschke the epipharynx in repose reaches to the hypopharynx and closes the mouth.'

From my observations I found that this structure viewed from the dorsal surface of the head in cleared preparations is more or

less flask-shaped when in the extended position. Its base rests on the clypeo-labral suture and from its distal end projects a large number of bristles of moderate length, which are closely aggregated together, giving it a very dark appearance. On the ventral surface it is connected on either side with the medial parts of the hair-bearing plates of the feeding-brushes. This surface is clothed distally with long fine hairs, some of which have branching ends projecting towards the mouth cavity. The posterior part of the ventral surface has on it a slightly raised oval plate with a peculiar arrangement of spines and hooks. This sclerite is the epipharynx and is placed above and in front of the mouth opening. The anterior part of the epipharyngeal sclerite bears on each side about six strong bristles which point downwards and medially from a transverse shaft. The bristles of one side converge and overlap each other as well as those of the other side. Posterior to the bristles is a strongly-chitinised hook-like structure from the lower end of which project several hairs. In addition to this armature the epipharynx bears numerous minute spines and fine hairs.

A pair of muscles arising from the middle line of the roof of the head gains insertion on the epipharynx posterior to the armature on a transverse line of chitin which passes along the lower margin of the epipharyngeal sclerite.

The Feeding-brushes (Fig. I and Fig. III). In spite of the elaborate descriptions of the different ways in which the brushes work, none of the previous writers on this subject has described their anatomy in any detail. Nuttall and Shipley (1901) write: ' On the extreme anterior end of the head are the two closely-packed bundles of slightly curved fine, hair-like setae which we have throughout this paper called the brushes. Other observers called them the rotatory, whirling and vortex organs, a name which suggests that the individual hairs move in succession and create a whirlpool. This is, however, not the case. All the hairs move together, and both organs move as a rule simultaneously and in the following way. The hairs are arranged in a sort of cupped fan-like shape, something like a very strong, overhanging, arched moustache. Their point of origin is attached to the extreme anterior edge of the head; and this portion of the head, the " clypeus " of Meinert, is hinged on to the rest of the head; the whole organs can be bent

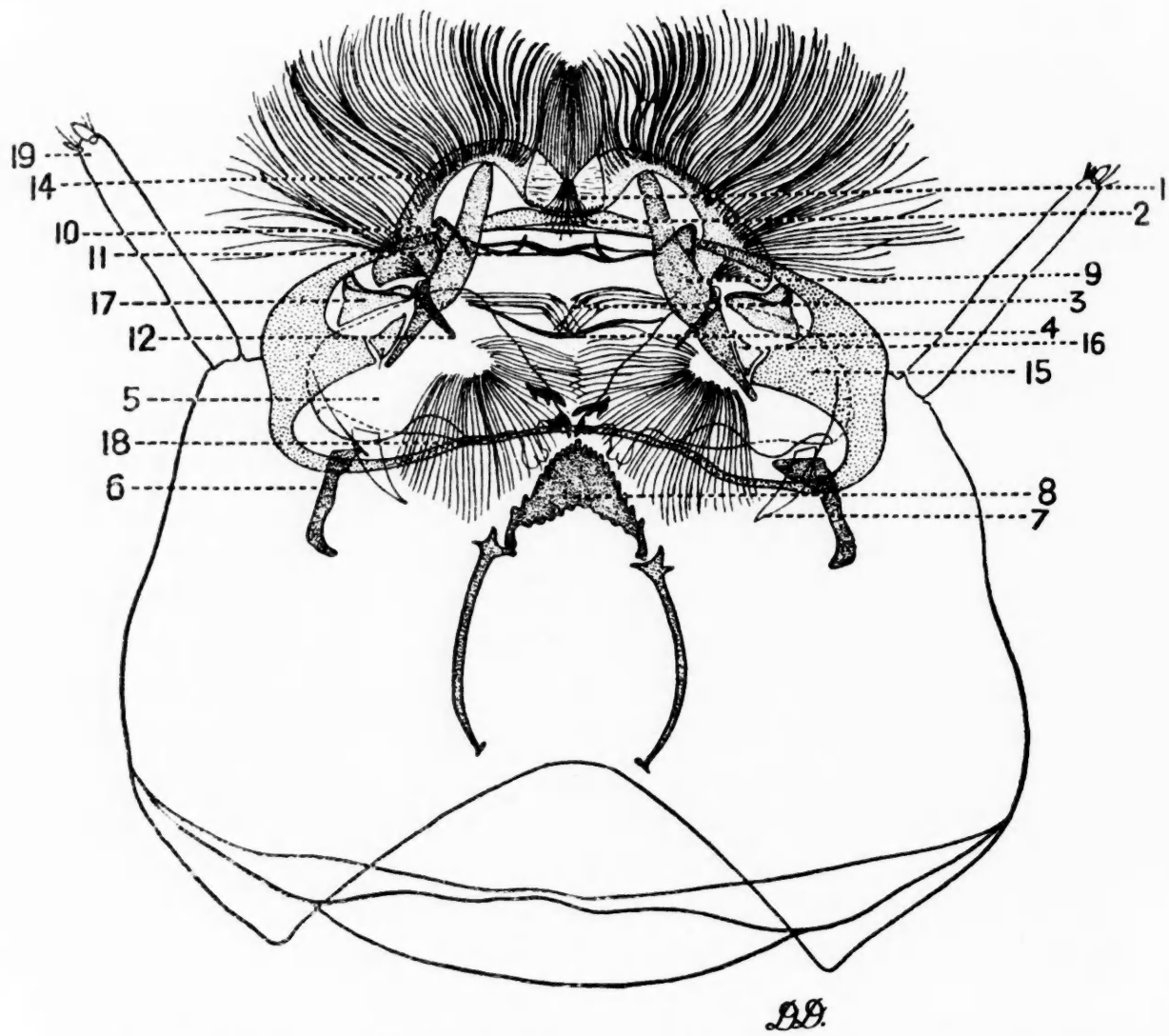

FIG. I. Head. Ventral view. Mandibles dotted. (1) labrum; (2) clypeus and clypeolabral suture; (3) epipharynx; (4) chitinous bar along the posterior margin of epipharynx; (5) mandible; (6) apodeme of mandible; (7) first segment of maxillary palp; (8) mental plate; (9) apodeme of feeding brushes; (10) lateral process of apodeme; (11) transverse chitinous plate connecting the two apodemes; (12) medial process of apodeme; (13) knob-like process of apodemes; (14) hair-bearing sclerite; (15) lateral plate on ventral surface of head for articulating the feeding brushes with the cranium; (16) lower triangular plate; (17) upper process of (No. 15) for articulation with knob-like process of apodeme and base of hair-bearing sclerite; (18) chitinous bar connecting lateral plates of head posteriorly and on which the labium and hypopharynx rest; (19) antenna. (= × 90).

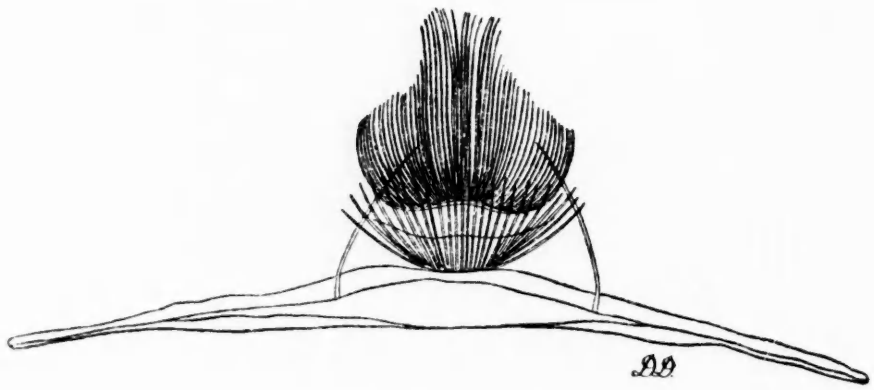

FIG. II. Labrum from the dorsal surface. (= × 160).

on this hinge, and when bent the two bunches of hair also tend to come together towards the middle line. The two brushes, however, can move independently, and at times one is seen bent under while the other remains erect.'

Thompson (1905) writes: 'The flabellae are protuberances, densely clothed with fine hairs and having in addition a peculiar arrangement of long yellow setae. Black-pigmented apodemes that are continuous with the black spot areas enter each flabella

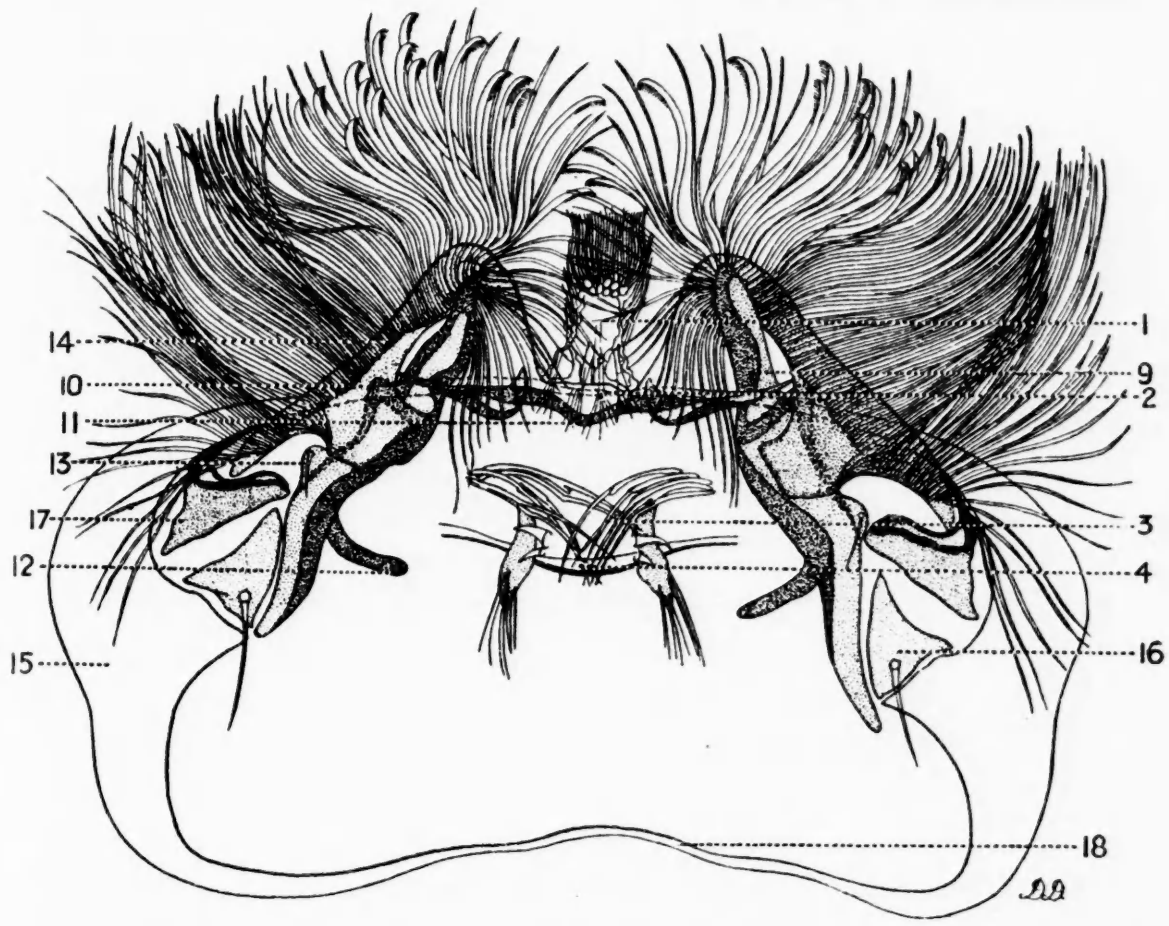

FIG. III. Feeding brushes, labrum, epipharynx as seen from the ventral surface of the head. Enlarged to show different parts. Explanatory numbers as in Fig. I.

and the two flabellae are united by a transverse rod. To the apodemes the flabellal muscles are attached, two for each flabella, an inner and an outer, both retractor in function and acting simultaneously. When these muscles contract the seta-bearing area of the flabella is depressed and the setae come together in a brush whose tip points caudad. On their relaxation the flabellae protrude beyond the front of the head and the setae stand out in great yellow fans. The

median prominence or palatum passively follows the movements of the flabellae which are strictly synchronous as far as I have observed.' The following account is an attempt to describe that part of the anatomy of these structures which has not been dealt with by previous writers, and in this way facilitate the further study of their mode of functioning.

The feeding brushes lie on either side of the ventral surface of the anterior end of the head. Each brush consists of a group of many long curved bristles closely attached in rows along the margin of a chitinous plate. The bristles nearer to the middle line are shorter and stouter than the outer ones which are longer and finer. The former are, moreover, armed distally with a row of fine teeth giving them the appearance of a comb. In predaceous Culicine larvae such as those of the genus *Lutzia* these teeth occur on all the bristles and spread down for a considerable distance. The presence of these teeth is interesting because the larvae of *Stegomyia fasciata* are not predaceous in habit.

Each feeding brush consists of the following parts :—

(1) A heavily pigmented rod-shaped apodeme (9) Figs. I and III.

(2) A hair-bearing sclerite external to it (14) Figs. I and III.

(1) *The Apodeme of the Feeding-Brushes.* This is a complicated rod-shaped structure which extends from the anterior end of the ventral surface of the head to the level of the middle part of the lateral border of the mandible, when it is in the adducted position. For purposes of description it will be convenient to divide it into three parts :—

(*a*) *The anterior third* articulates distally with the upper end of the hair-bearing plate. This end fits into the arch formed by the junction of the sclerite (14) Figs. I and III, with the labrum (1). The proximal end of this part of the rod possesses a strong laterally directed process (10), which fits into the concave medial surface of the sclerite (14). Medially the two apodemes are joined to each other at this level by a transverse plate (11), the two plates joining in the middle line. Each of these plates has its lower edge markedly upturned on either side of the middle line. The two plates serve to unite the two brushes and synchronise their movements.

(*b*) *The middle third* is the most dilated and heavily chitinised

part of the apodeme. On the middle of its dorsal surface is a strongly chitinised area which is joined by a curved, stout rod to a similar area at the lower medial end of this surface, the curved rod afterwards projecting on the medial side of the upper end of the posterior third of the apodeme as a strong process (12).

(1) *The posterior third* of the apodeme ends in a pointed curved process which lies dorsal to the middle of the lateral border of the mandible, when the latter is in the adducted position. At its upper end this part has the process (12) on its medial side and bears laterally a small knob-like process (13) which articulates with an upper, somewhat triangular process (17) connected with the hair-bearing sclerite. It is interesting to note that a short bristle arises from the process (13). On the medial side of the process (12) a narrow transverse bar of chitin passes along the posterior margin of the epipharyngeal sclerite to end on the medial side of the process of the other side.

(2) *The Hair-bearing Sclerite* (14) Figs. I and III. This sclerite is situated on the lateral side of the apodeme. Each sclerite is somewhat triangular in shape. The lateral border is markedly convex and carries the hairs of the brush. It unites distally with the medial border to form the apex of the triangle, and then it curves over the apodeme to join the labrum. The posterior part of this border joins the base medially and becomes connected on its lateral side with the lateral end of the plate (17) which lies immediately below the base. The latter curves acutely inwards from the lateral border to join the medial one dorsal to the middle of the apodeme. At this junction the chitin exhibits a peculiar striated appearance. The medial border is not straight but its upper part is curved and its lower part almost straight and is in close contact with the dorsal surface of the apodeme. The medial side of the plate is concave in its upper part, and in this concavity fits the lateral process (10).

THE ARTICULATION OF THE FEEDING-BRUSHES WITH THE HEAD CAPSULE

The posterior third of the apodeme and the curved base of the hair-bearing sclerite, articulate on either side with the medial margin of that part of the ventral surface of the head anterior to the bases of the antennae. This part is strongly chitinised and broader

posteriorly than anteriorly. Between the lower third of the apodeme and the inner margin of this surface is a small V-shaped space which is almost completely occupied by a small triangular plate of chitin (16) from the lower medial angle of which projects a strong bristle. The upper margin of the V-shaped space forms the lower border of a plate of chitin (17). The upper surface of the latter is grooved and articulates medially with the knob-like projection of the apodeme (13). The pointed lower, lateral angle of the hair-bearing plate fits into this surface near its centre. The lateral margin of the plate (17) is joined to the lateral border of the hair-bearing sclerite as already stated. Anteriorly the articulating plate becomes thinner by the approximation of its margins, and the two plates join each other across the middle line to form the clypeo-labral suture (2). Posteriorly again they are joined by a transverse bar of chitin which is markedly thinner in its central part. This bar (18) passes immediately below the mandibles and dorsal to its apodemes. Attached to the central part of this line and directed posteriorly is a plate of thin chitin on which rests the labium and hypopharynx.

The muscles which move the brushes are two pairs, one external and another internal. The external takes origin from the dorsum of the head behind the antenna, and passes forwards to gain insertion on the distal lateral dark area on the dorsal surface of the apodeme. The internal muscles arise on each side of the middle line of the posterior margin of the dorsum of the head capsule, and pass forwards near to the muscles of the opposite side and are inserted on the curved rod below and medial to the external muscles.

From this arrangement it seems that the internal muscles act as flexors and the external ones as extensors to the feeding brushes. The distal transverse plate which joins the central apodemes serves to synchronise the movements of the two brushes. The whole apparatus (apodemes) probably represent a specialised form of the tormae. (Patton and Evans. 1929.)

The Maxillae (Fig. IV). Each maxilla is an almost triangular piece of chitin. It possesses a lateral margin along which runs the maxillary palp, a medial margin which runs upwards and outwards to meet the lateral margin near the upper end of the palp. On the upper part of this margin is a long tuft of bristles, below this is

a row of several stout, shorter bristles. From the posterior end of the lateral margin the base first runs nearly vertically upwards and then joins the medial border by a transverse line, giving the maxilla a pointed appearance posteriorly. A chitinous ridge (1) runs obliquely on the dorsal surface, on the anterior part of which project two stout bristles. The inner edge of each maxilla approaches its fellow of the other side but does not touch it ; in the

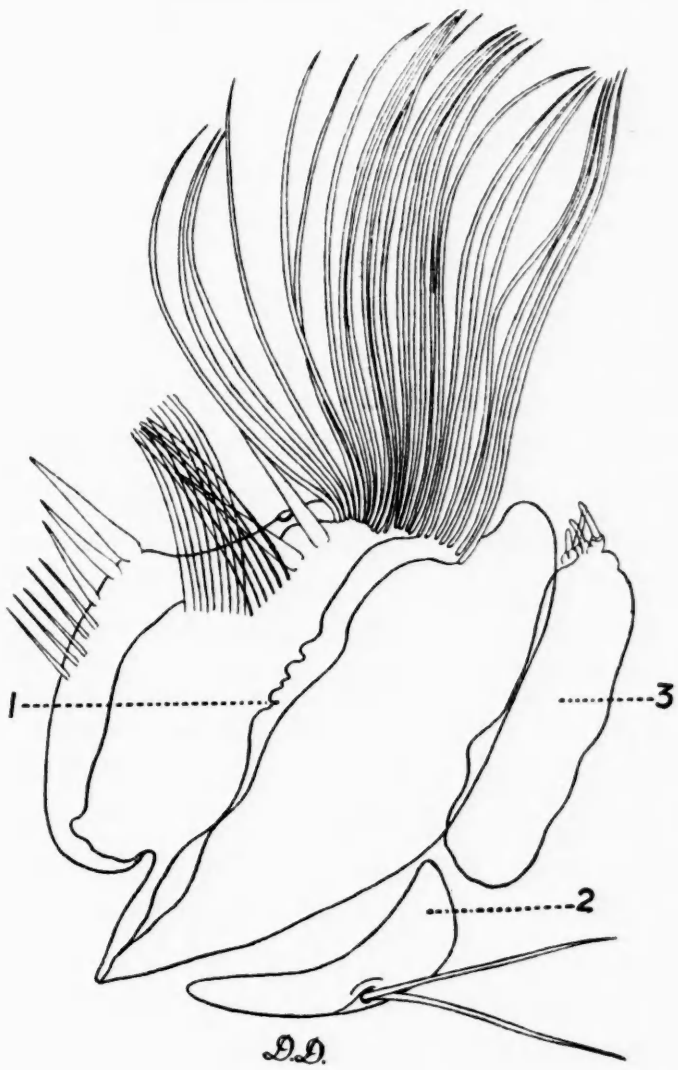

FIG. IV. Maxilla. Note the chitinous thickening on the dorsal surface (1). (2) first segment of maxillary palp with its double bristle ; (3) second segment of palp. (= × 240).

angle between the two lies the triangular toothed labial plate Each maxilla bears along its outer margin a palp which lies in a plane slightly dorsal to the maxillae. This palp is a large stout structure and consists of two segments, the lower one (2) is curved downwards

and inwards, and bears near the middle of its outer edge a paired bristle (two bristles from one socket). The second segment (3) is almost double the size of the first one and of almost conical shape and extends as far as the junction of the lateral and medial borders of the maxilla. It bears at its anterior end four stout, short bristles. The maxilla is united to the head by a wide joint and shows but little movement in comparison with the mandible. Each receives a pair of muscles which arise from the ventro-lateral surface of the head capsule and are inserted into the middle of the base of the maxilla.

The Mandibles (Fig. V). Each mandible is roughly quadrilateral in shape. It consists only of one segment and forms the sides of the mouth cavity, the floor of which is partly formed by the maxillae. It articulates by its base with the ventral surface of the head. Its lateral margin is convex. The medial margin is toothed, and together with the same border of the mandible of the other side is opposed to the strongly toothed labial plate. From below upwards the following structures occur on the medial border :—(1) Lightly chitinised finger-like process on which there are some fine hairs and which has been homologised with the mola of the mandible of the cockroach (Patton and Evans. 1929). (2) Above this two strong composite teeth. The lower one is on a more dorsal plane than the upper. The former has three teeth and the latter four. Immediately above the latter tooth are two stout curved bristles, the lower of which has a distinct serrated margin. At the extreme anterior end of the medial border are two long very stout bristles which have been homologised with the palp of the mandible of Crustacea (Patton and Evans. 1929). Behind the latter is a shorter bristle and a little raised area of chitin. Between the upper bristles and the teeth is a row of moderate sized stout bristles. From this it can be easily seen that the mandibles are admirably adapted for combing out the mouth brushes and sweeping the particles of food collected by the brushes into the mouth, the teeth being used for breaking the larger particles, as these teeth and the teeth of the submentum are the only crushing apparatus the larva possesses.

Each mandible has attached to its lower external angle a strong short chitinous apodeme which passes downwards to join the posterior part of the ventral surface of the head above and medial

to the eyes. This is very similar to the *trochantin of the mandible in Orthoptera*. Each mandible is moved by a pair of stout muscles. It receives an abductor on its lower external angle and an adductor on the lower internal angle. These muscles arise by triple heads a short distance behind and below the eyes, the fibres of each group converging as they pass to their insertion.

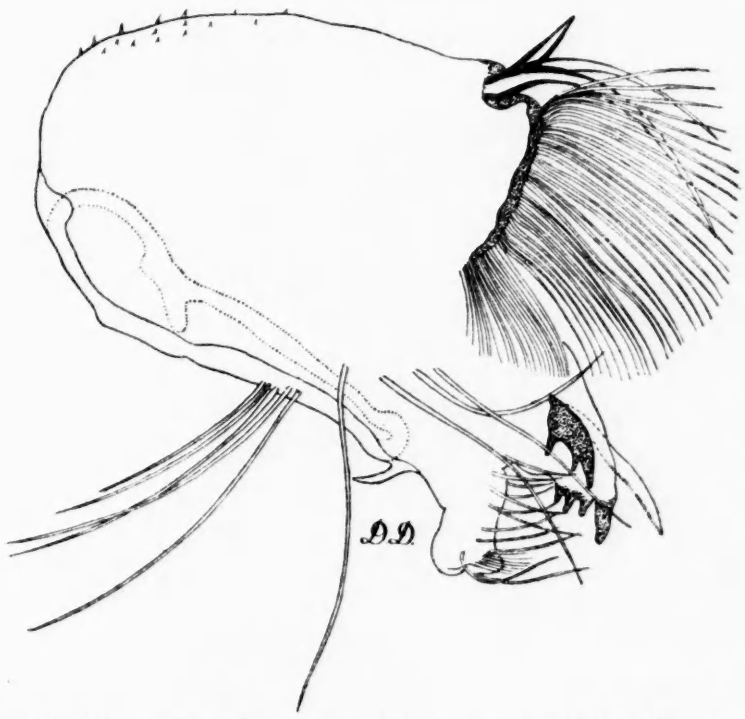

FIG. V. Mandible. Note the teeth and bristles on its medial border. (= × 240).

The Labium (Figs. VI and VII). This structure is a complicated one and consists of the following parts ventro-dorsally :—

(1) The most ventral structure; a delicate fringe of hairs situated on a chitinous crest (Fig. VI, 1).

(2) A densely chitinous, comb-like, triangular plate with one median long tooth, and from eleven to thirteen smaller ones on either side, the lower ones on the plate being more separated from each other than the upper ones (Fig. VI, 2).

(3) Dorsal to this triangular plate and separated from it by a little space into which the teeth of the mandibles fit in the adducted position, is a more lightly chitinised plate (Fig. VI, 3, and Fig. VII). This plate is nearly oval in shape. Its anterior margin has two short, blunt processes, one on either side of the middle line, and between the two are several minute spines. Below these processes the

margins of the plate have two rows of teeth, an anterior row and a posterior one. The former has four to five unequal teeth and the latter about twelve smaller teeth which extend to the margin of the hypopharynx. Between the rows of teeth of both sides the body of the plate bears peculiar peg-like structures. The fore-edges of this plate and the more ventral dark one form the hinder border of the mouth opening.

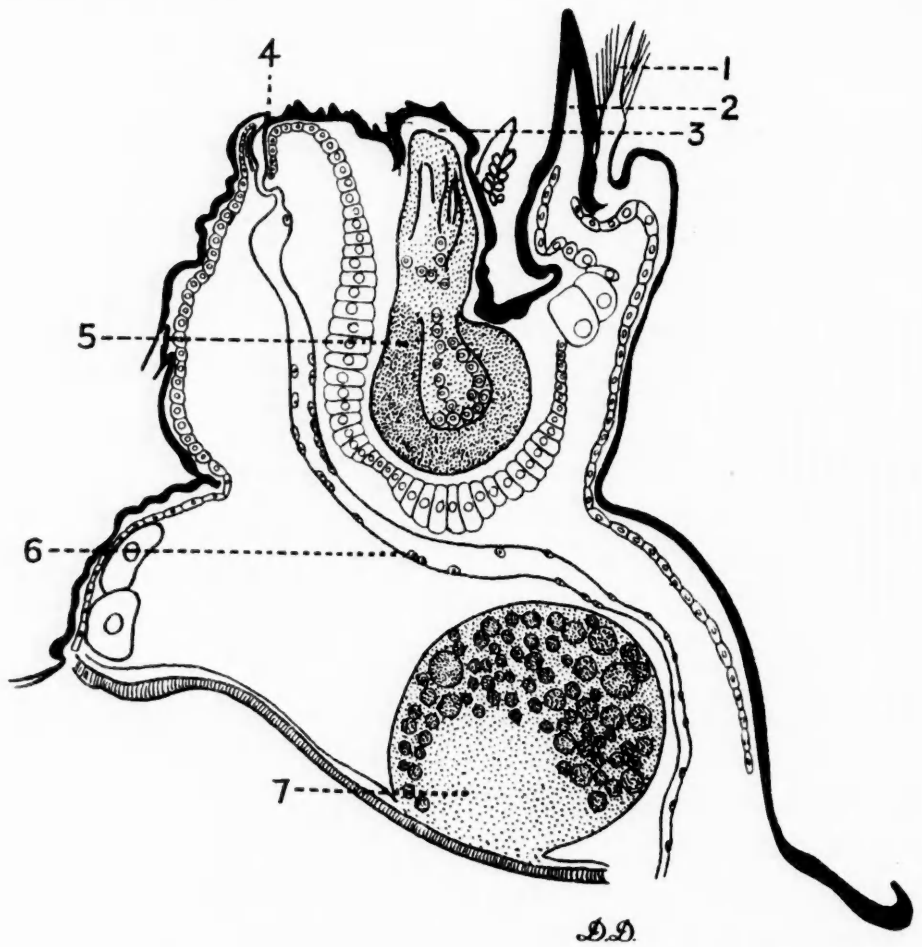

FIG. VI. Longitudinal section of the posterior part of the floor of the mouth, to show the arrangement of the different parts of the labial plate, the course and opening of the common salivary duct. (1) plate with the hair fringe; (2) tooth of the mental sclerite; (3) labium, in part; (4) hypopharynx; (5) imaginal bud of labium; (6) common salivary duct; (7) sub-oesophageal ganglion. Note the dilatation of the distal portion of the salivary duct before its opening on the hypopharynx, and note the curve of the posterior lip of the latter. (= × 320).

(4) Still more dorsal to this plate is the hypopharynx with the opening of the salivary duct.

It is extremely difficult to arrive at the exact homology of these different parts, but before expressing my opinion about their probable homology I think it would be of great help to quote what previous authors have said about it.

Miall and Hammond (1900), describing similar structures in the larva of *Chironomus*, say : ' The maxillae of the second pair, which often unite to form a single organ, *the labium*, can only be understood by comparison with other insects. In the Chironomus larva they have lost so many of the original parts that at first sight they seem to consist of a single comb-like plate, whose teeth point forwards, and are opposed to the mandibles, helping them to grasp or divide the food. On close examination a second plate is discovered above the other, and almost hidden by it. The upper plate is of softer texture, and furnished with many spines and bulb-like projections, some of which may be connected with the sense of taste. The fore-edges of these two plates form the hinder border of the mouth-opening. In Orthopterous insects, which with respect to the mouth parts are less specialised than most others, there are two successive plates at the base of the labium, a basal and larger piece, called the *submentum*, and a distal piece, *the mentum*, to which the terminal parts are attached. It seems to us probable that the mentum of Chironomus larvae has gradually slipped behind the submentum, which now almost completely conceals it.'

Later, Thompson (1905), in his description of the larva of *Culex*, wrote : ' The mental sclerite was called " under lip " by Meinert and corresponds to the structures figured by Miall and Hammond for larvae of *Chironomus*, Miall for *Dicranota*, and Miall and Walker for *Pericoma* as " submentum." Raschke identified the fold that I have termed the transverse line with the mentum, " Kinn." For Miall, the sclerites which correspond to the saddle-shaped sclerite of *Culex* are " mentum." With *Culex*, however, the sclerite sheaths the bud which forms the labium of the adult and is to be regarded as the larval labium. The mental sclerite would seem to be either a mentum or a submentum, as its hypodermis passes into the floor of the head.'

Imms (1907), in his description of the same structures in the larvae of *Anopheles*, said : ' Between the two maxillae there lies a pointed toothed plate. It is termed by Meinert the " under lip " and by Felt (1904) " the labial plate " ; the latter writer figures it for the larvae of a large number of North American Culicidae. This sclerite is most probably to be regarded as the representative of the second maxillae which have united to form a single organ. A similar

plate is often found among Dipterous larvae, and usually possesses a second sclerite lying immediately above it. This is the case in *Chironomus, Dicranota, Phalacrocera,* and *Pericoma.* The lower plate is regarded by Miall and Hammond (1900) as the " submentum " and the upper one as the " mentum." They believe that the mentum has gradually slipped behind the submentum so that the latter completely hides it when viewed from below. In *Anopheles* the " labial plate " corresponds with the " submentum " of the *Chironomous* larva ; it bears on its upper, or pharyngeal surface, a considerable array of strongly chitinised teeth and ridges, but I have not been able to homologise any of them with tolerable certainty with a " mentum ".'

Dyar and Knab (1912), in their description of the mouth parts of the larva of *Culex,* wrote the following : ' At the attachment of the maxillae the hypocranium terminates in a sharp transverse ridge, somewhat lobed outwardly from the maxillae. Behind this ridge, the parts supporting the mental and labial structures turn abruptly to the hypopharynx and pharyngeal opening. Medially, and immediately behind the ridge, are four successive structures. In a ventral view these are in order. (1) A delicate roughly triangular plate with a marginal hair fringe and produced into a long tooth at the middle ; behind this, (2) the mental sclerite, a heavily-chitinised dark, roughly triangular dentate plate ; and back of this, (3) a complicated plate with teeth and spine-like structures, the labium ; further back, at the margin of the pharynx, is the hypopharynx, a simple chitinous cone. The first two plates are excrescences of the mentum ; the labium is attached to the mentum at the inner side by a flexible membrane. The labium, though complex in structure, allows none of the parts of that organ to be recognised, it presents very different aspects in dorsal and ventral view. It can be drawn back by two muscles attached to its outer angles.'

I. M. Puri (1925) described the labium in the larvae of *Simulidae* as follows :—' The labium lies ventral to the hypopharynx and forms the anterior and ventral border of the salivary slit. At first sight the plate lying immediately below the opening appears to be a part of the hypopharynx and has erroneously been described as such by Johannsen, Pomeroy and others. But on comparing the

whole structure (hypopharynx and labium) with that found in the larvae of certain other Diptera, e.g. *Chironomus* (Miall and Hammond, 1892), *Anopheles*, *Culex* (Thompson, 1905, and Howard, 1912), and *Ptychoptera* (de Meijere, 1916), and of some Coleoptera, it seems obvious that the parts lying in front of the salivary opening are portions of the labium shifted backwards. The labium is composed of three parts: (1) A strongly chitinised anterior or dorsal plate, the *prementum*, (2) passing backwards into a soft plate, the Mentum; and (3) a hard-toothed, ventral plate, the *submentum*.

The prementum is a double plate forming the floor of the salivary chamber and bears a pair of broad palp-like structures having a number of cup-shaped projections each of which is provided with two jointed sensory papillae. Between these sensory structures there is a pair of strong chitinous spine-like projections on each side of the middle line. The prementum bears a large number of long branching setae along its anterior border and a comb of short stiff ones lying dorsal to the palp-like structures. The ventral wall of the prementum is strengthened by a chitinous thickening on each side and passes backwards into the soft thin *mentum*. The *submentum* (wrongly called the " labium " by many writers; some call it the " mentum ") is the hard chitinous plate lying ventral to the prementum and mentum, and is fused to the head capsule only along its toothed border. It bears nine strong teeth in the front row (the median tooth and the one at each end much larger than the other six), and a pair of smaller teeth external to the large teeth of the side. Just below these teeth the head capsule is produced into five broad, thin projections. Ventrally in the region of the submentum, the head capsule bears on each side four to five long sensory hairs, some of which have bifid ends.'

The nomenclature here provisionally adopted follows that of the majority of writers on larval taxonomy, who describe the triangular toothed plate as the *mentum* and *mental plate*. The oval softer more dorsal plate then becomes that part of the labium distal to the mentum, and the most ventral fringed plate may be regarded as *submentum*. Fig. VI represents a longitudinal section of the head and shows the arrangement of the different parts, and it will be noticed that immediately below the teeth of the third oval plate

is an extremely prominent, paired structure, projecting from the bottom of a wide pocket, the two sides of this structure being confluent at their base. This structure is the imaginal bud of the labium (5), and as can be seen from the figure it has no connection with the toothed triangular plate. Therefore, as far as my observations go, it would seem that the oval soft plate constitutes the main part of the labium and possibly even includes the mentum as stated by Miall and Hammond, and Puri. If this interpretation is the correct one, the toothed plate must represent the submentum,

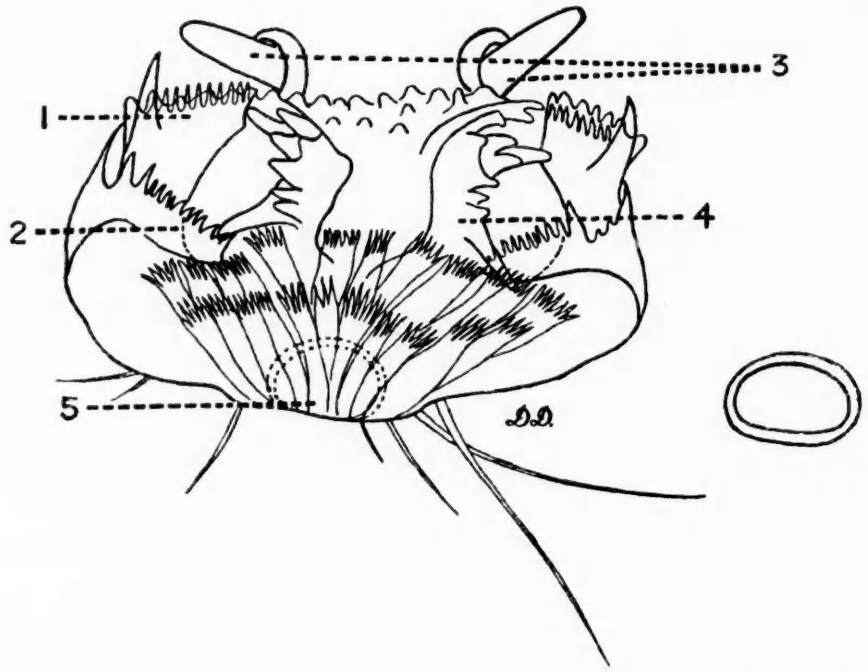

FIG. VII. Labium seen from above to show the arrangement of the teeth and the hypopharynx. (1) upper row of teeth ; (2) lower row of teeth ; (3) blunt anterior processes ; (4) peg-like processes ; (5) hypopharynx with the opening of the salivary duct. Note the strong branching hairs on the latter. (= × 700).

and then the interpretation of the fringed plate becomes difficult. As, however, the term ' mental plate ' is employed generally in taxonomic literature, it would seem that further study of the development of these structures and the morphology of the adult labium is necessary before making any definite change in the terminology. The labial plate is supplied with a pair of muscles which run from the divergent linear thickenings behind the plate and are inserted on the hinder angles of it. They are depressor in action.

The Hypopharynx (Fig. VI, 4) is a minute conical projection which lies immediately dorsal to the labium. On the top of this

cone is the opening of the salivary duct. The lips of the opening bear several stout hairs. The posterior lip of the hypopharynx is markedly arched and bends forward over the duct opening so that the direction of the opening is towards the mouth cavity. Under the hypopharynx are a few scattered muscle fibres.

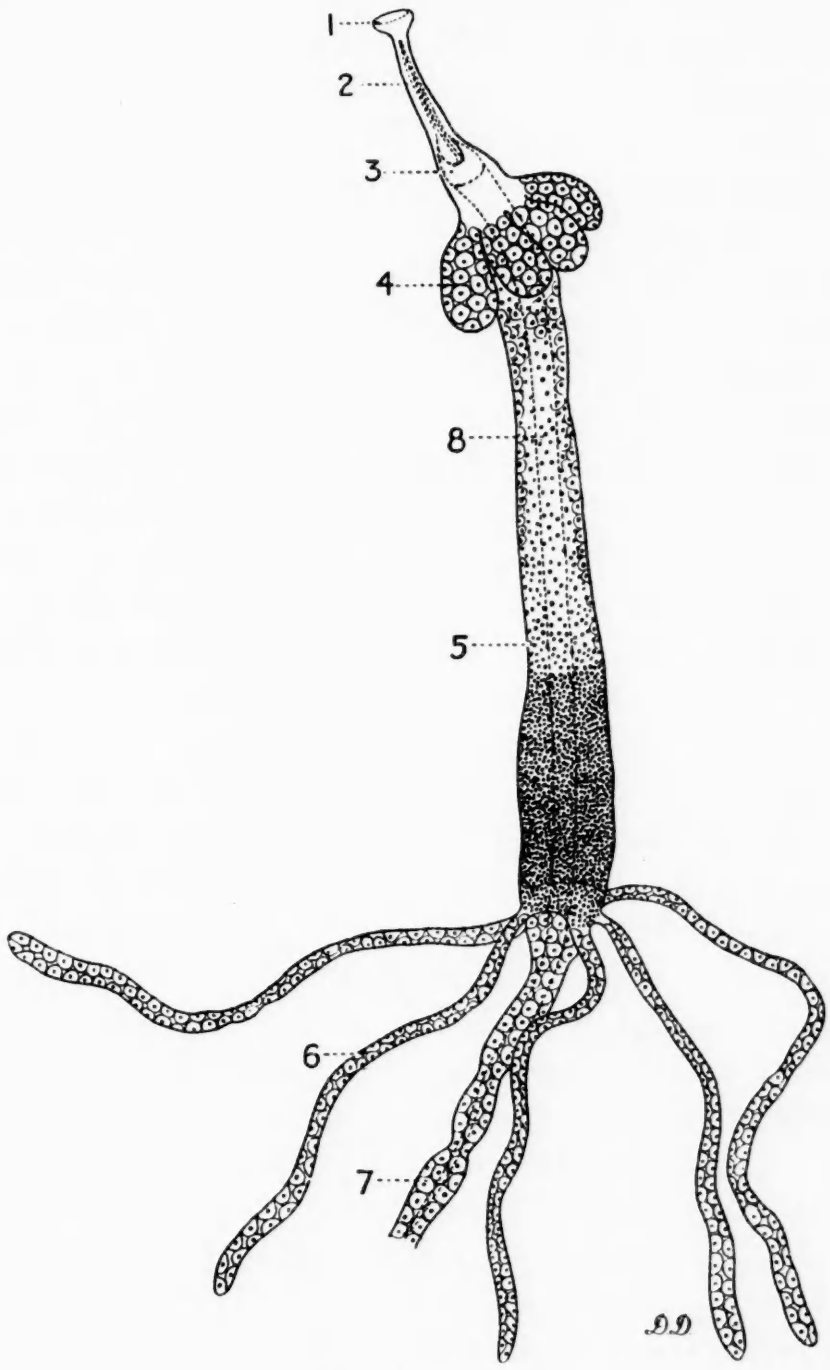

FIG. VIII. Alimentary canal dissected out. (1) pharynx; (2) oesophagus; (3) proventriculus; (4) gastric caeca; (5) mid-intestine; (6) malpighian tubules; (7) hind-intestine; (8) peritrophic membrane attached above to proventriculus and reaching down to the lower end of hind-gut. (= × 20).

THE FORE-INTESTINE AND ITS APPENDAGES

For descriptive purposes the fore-intestine consists of the following parts :—(1) Mouth cavity ; (2) Pharynx ; (3) Oesophagus ; (4) Proventriculus. The appendages belonging to the fore-intestine are the salivary glands.

Mouth Cavity. The mouth cavity lies between the mouth parts. The epipharynx forms its roof. Behind the *epipharynx* is another folding of the roof of the mouth projecting downwards and containing some transverse muscle fibres, presumably the *post-epipharynx.* The *mandibles* form the sides, and the *maxillae* with the *labial plate* the floor. The side walls are further completed by the overlapping of the hairs of the feeding brushes, and also by the hairs on the mandibles, which meet those of the brushes. These structures have been already described.

Pharynx (Fig. IX, 1). The pharynx is a large chamber occupying most of the head. Its walls consist of a chitinous intima continuous with the general cuticle of the body wall and resting on a layer of epithelium. External to the epithelium is a strong layer of muscles. At its commencement the pharynx is flattened dorso-ventrally and has a greater transverse diameter than in the succeeding parts. It has a ventral and a dorsal wall, which have been described by Imms (1907) as the lateral crests, and a roof. At the posterior end of the pharynx the lateral crests come near each other and finally meet in the middle line in front of the beginning of the oesophagus. The chitinous layer of the roof is considerably thickened on either side and forms a pair of rigid plates. Traced backwards these plates become narrower and finally terminate just anterior to the union of the lateral crests of the pharynx. External to each chitinous plate is a rod-like thickening of the intima which serves to give a firmer attachment to the pharyngeal muscles. Situated on the crests of the pharynx is a row of bristles which are also present on the roof.

The pharynx has an elaborate musculature. The crests are united to the wall of the head near their posterior ends by stout muscles. The hinder part of the roof also provides insertion for two muscles which probably act as elevators to the pharynx. The crests also possess a well-developed circular coat of muscles which are principally developed in relation to the posterior part.

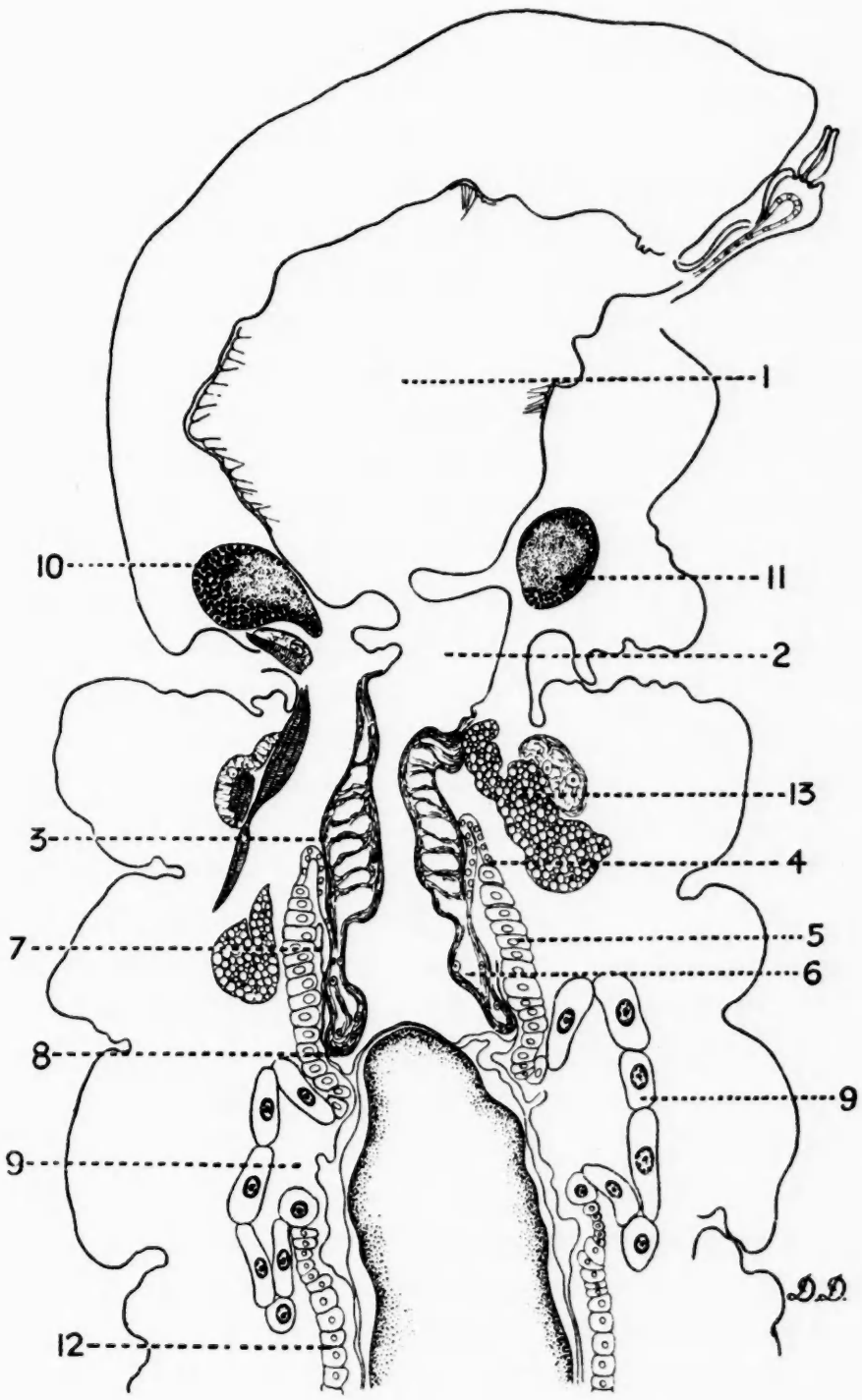

FIG. IX. Longitudinal section of head and thorax. (1) pharynx; (2) oesophagus; (3) circular muscle layer of oesophagus; (4) point where the oesophageal epithelium terminates and the proventriculus cells begin; (5) cells of proventriculus; (6) blood space at the lower free end of oesophagus; (7) peritrophic membrane attached to proventriculus; (8) chitinous intima at the free end of oesophagus to show its thickening; (9) gastric caeca opening into (10) mid-intestine; (11) sub-oesophageal ganglion; (12) supra-oesophageal ganglion; (13) fat cells. Note that the peritrophic membrane is not reflected into the cavities of the gastric caeca. (= × 90).

Oesophagus (Fig. IX, 2). Between the supra-oesophageal and sub-oesophageal ganglia the pharynx becomes continuous with the oesophagus. The latter is a tube of simple structure which passes through the neck and joins the proventriculus in the thorax. It is prolonged into the cavity of the latter for some distance as an inner tube and then becomes reflected on itself, passing upwards to the top of the proventriculus and finally bends downwards and immediately joins the cells of the proventriculus.

The oesophagus is not a tube of uniform diameter throughout. The part in the neck is dilated owing to the fact that the ventral wall possesses a sort of large cul-de-sac. The dorsal wall has also a smaller one immediately below the commencement of the oesophagus. This appearance may be an artifact due to the sudden killing of the larvae by dropping them into warm Bles's fluid, but it has been observed in all the larvae. The thoracic part passes down immediately into the proventriculus. The chitinous intima of the oesophagus is strong and markedly corrugated. The epithelium under it is of the flat-celled type, and external to this is a well-developed muscular coat consisting of a layer of circular muscle fibres. Beneath the circular muscle layer lies a longitudinal series of muscles which certainly function as dilators. In the region of the proventriculus the chitinous intima and the epithelium of the oesophagus are the only parts of the latter which become reflected, the circular muscle coat ceases some distance before the lower bend is reached. Above the free lower end of the oesophagus there is an empty space often referred to in the literature as a blood space. Imms (1907), in his description of the *Anopheles* larva, wrote: 'The space between the inner and downwardly directed wall and the outer or reflected wall of the oesophagus contains a good deal of blood and there is also a circular sinus situated between the muscular layer and the inner wall. This structure surrounds the gut in the form of a complete ring; it is covered by a layer of connective tissue, and its interior shows a spongy structure, its cavity being much intersected by strands of fibrous nature. It has been found in several dipterous larvae, and is regarded as being a blood sinus.' The description given by Thompson (1905) for the larva of *Culex* also agrees with this. He says: 'At the free border of the oesophagus the space between the inner or direct face and the outer or

reflected face is occupied by a blood sinus.' Miall and Hammond (1900) in *Chironomus*, and Saunders (1924) in *Forcipomyia* have described a blood space of varying size, between the direct and reflected walls of the oesophagus, but Puri (1925) in his description of *Simulium* says that ' The valve of *Simulium* differs from those of other Nematocera in that it has no extensive blood space.'

Wigglesworth (1930) has described a similar sinus in the saw-fly larva and in the adult of *Bombus*. He also figured it in the oesophageal valve of *Ceratophyllus wickhami*, in the larva of *Aeschna* (Odonata), and in *Blatella germanica* (Orthoptera).

Above the blood space is a well-developed coat of circular muscle fibres. Delicate oblique fibres pass from the oesophagus across the shoulder of the proventriculus thereby helping in the attachment of both structures to each other. These fibres are evidently not muscular. In transverse sections the arrangement of the different layers near the commencement of the oesophagus are as follows from within outwards :—

(1) A well-developed chitinous intima, sharply folded and appearing rosette-like.

(2) An epithelial layer consisting of a single layer of cells ; the latter are generally inconspicuous but can always be made out by the presence of nuclei.

(3) A basement membrane on which the epithelial cell layer rests. This is also thrown into longitudinal folds which alternate with the folds of the chitinous intima.

(4) A circular muscle layer which is large and conspicuous. Outside this and restricted to the part of the oesophagus above the proventriculus is a layer of longitudinal muscles.

Proventriculus. The proventriculus is a specialised part of the fore-intestine, and is structurally an invagination of the oesophagus into the mid-intestine. Wigglesworth (1930) says : ' At this point the fore-gut, the oesophagus, is invaginated into the mid-gut to form the structure usually referred to as the " oesophageal valve," an unfortunate term that attributes to this part of the intestine a property it does not possess ; for the invagination acts not as a valve but as a sphincter.' The lumen of the oesophagus, as it passes down, gradually widens. It carries with it the chitinous intima as well as the epithelial and circular muscle

layers. The chitinous intima and the epithelial layer pass down to the free lower end of the oesophagus and are then folded back upon themselves. The space between the reflected layers above the lower end is a blood space, as the circular muscle coat ceases a little lower than half-way down the descending part of the oesophagus. The greatly attenuated epithelium gradually develops into large columnar cells of open fibrous cytoplasm with large nuclei. At the upper bend of the oesophagus the cells again gradually decrease in size till they join the cells of the proventriculus. The beginning of

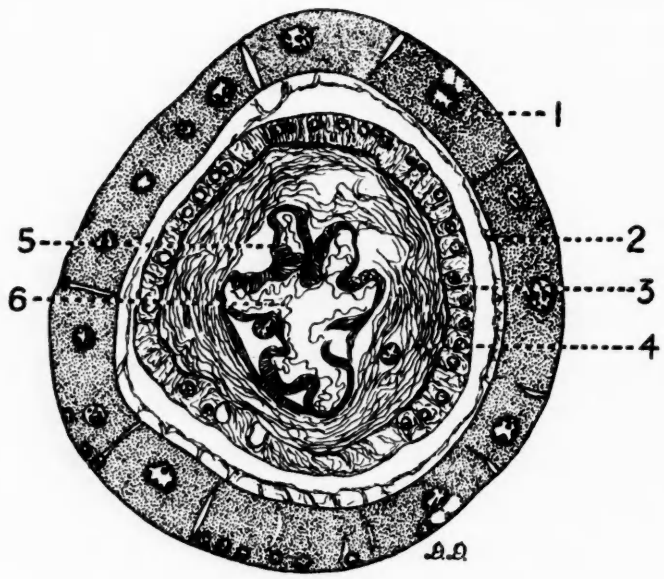

FIG. X. Transverse section at the upper part of proventriculus to show the arrangement of the different layers. (1) cells of proventriculus; (2) peritrophic membrane along the inner margin of proventriculus; (3) reflected layer of oesophageal epithelium; (4) circular muscle layer of oesophagus; (5) epithelial layer of the direct part of oesophagus; (6) chitinous intima of oesophagus. Note (*a*) the vacuolation and size of the proventriculus cells; (*b*) the character and shape of the reflected layer of oesophageal epithelium, compare it with the epithelial layer of the direct part as well as with the reflected layer in the next figure; (*c*) the absence of a reflected layer of chitinous intima; (*d*) the foldings of the chitinous intima surrounding the lumen of the oesophagus. (= × 240).

the latter cells is very abrupt. Both longitudinal and transverse serial sections demonstrate that the chitinous intima is not reflected round the entire structure and does not follow the epithelial cell layer the whole distance up, but distinctly ceases near the point where the cells of the epithelial layer gradually change their character, that is near the upper end of the blood space. The part of the chitinous intima covering the free margin of the oesophagus is remarkably developed and thicker than other parts and shows very slight corrugations. The muscular layer of the

oesophagus, as already mentioned, extends only a little more than half-way along its descending part and forms a strong circular band capable of closing the lumen.

In the larva of *Anopheles* as stated by Imms (1907), some stout longitudinal muscle fibres are present in the proventriculus. No

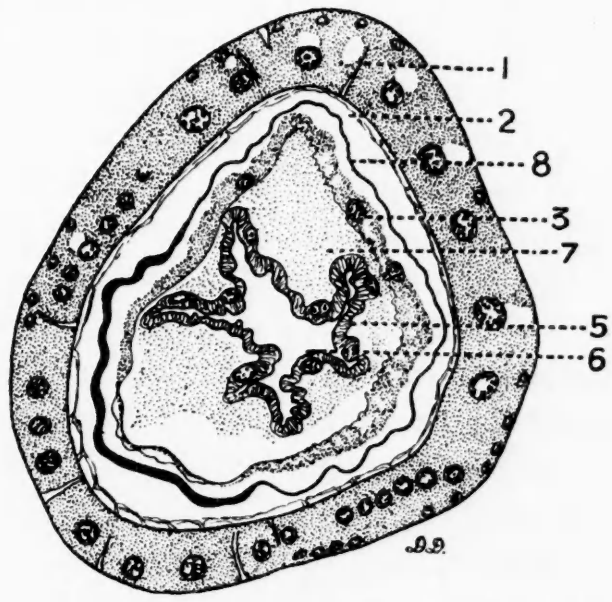

FIG. XI. Transverse section of proventriculus near its lower end. Explanatory numbers as in Fig. X. Note the disappearance of the circular muscle layer and its replacement by a blood space (7); also the presence of a reflected layer of chitinous intima outside the reflected layer of oesophageal epithelium. Note the thickening of the intima in its lower part, clearly seen in the figure. Compare the cells of the reflected layer of epithelium with the same layer in Fig. X. (= × 240).

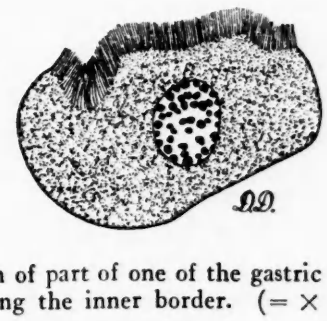

FIG. XII. Transverse section of part of one of the gastric caeca to show the size of the cells and the cillia-like arrangement along the inner border. (= × 400).

longitudinal muscle fibres have been found, however, in the proventriculus of *Stegomyia* larvae. This layer is also absent in *Culex*, and probably *Aedes*, as stated by Thompson (1905). There are no spines on the chitinous intima which Puri, in the larva of *Simulium*, states 'to be provided with festoon-like rows of short stiff bristles pointing backwards all round in the region of the bend.' He also states than Van Gehuchten has also described numerous hooklets covering a part of the reflected wall of the oesophagus in *Ptychoptera*

and thinks that ' their probable function is to enhance secretion in the cells of the cardia, which come in contact with this region. But that the function of these bristles in *Simulium* larvae is to help the passing backwards of the peritrophic membrane, as already suggested by Strickland, seems to be more probable.'

The proventricular cells proper begin abruptly a little below the upper and second bend of the oesophagus. The cells are large and of the columnar type and their cytoplasm stains deeply with haematoxylin. The nuclei are large and prominent and contain much chromatin. These cells frequently seem to exhibit vacuolation in their cytoplasm (Figs. X and XI). Further back they decrease in size and stain less deeply, and immediately below a point opposite the free lower end of the oesophagus, they abruptly join the cells of the *gastric caeca*. Externally the cells rest on a connective tissue layer and are lined on the inside by the peritrophic membrane. The latter structure extends downwards as a thin colourless tube for a considerable distance down the mid- and even to the hind intestine. This membrane forms a sort of lining to the food as it passes down the intestine offering protection to the delicate underlying epithelium.

Wigglesworth (1929), working on the nature of the peritrophic membrane in *Glossina*, has discovered that it is composed of chitin and is freely permeable to digestive enzymes and to haemoglobin. Further, he found that the membrane is formed by the condensation of globules of secretion into a solid sheet. The secreting cells of the membrane are the cells proper of the proventriculus. The secretion from these cells is drawn through an annular press and thereby moulded to form the peritrophic membrane. The circular muscle layer of the oesophagus will serve as a sphincter to close the upper part of the orifice and when the sinuses in the fore margin are distended they will serve to close the lower parts.

In sections of *Stegomyia fasciata* I have found that this membrane always takes origin from the upper end of the cells of the proventriculus or immediately below that point where it can be seen in favourable preparations attached to a point on the inner margin of one of these cells. As it passes down it becomes gradually separated from the epithelium by a space of variable width usually filled with granular fluid, most probably of a secretory nature. This membrane crosses the aperture of the gastric caeca but is never

reflected into their cavities. The space between the reflected portion of the oesophagus and the proventriculus does not contain any food.

The Mid-Intestine is a long cylindrical tube which occupies more than half the length of the alimentary canal. It is widest in front, gradually tapering to its junction with the hind intestine at the origin of the Malpighian tubules. The gastric caeca are glandular appendages belonging to the mid-intestine. They consist of eight subequal diverticula. The caeca of this larva do not present any constrictions as described by Imms (1907) for the *Anopheles* larvae. They consist of a single layer of large epithelial cells resting externally on a thin connective tissue membrane. The cells are subequal in size and show considerable intracellular material, consisting of coarse granules with great affinity for eosin. The nuclei are very large and prominent and contain numerous chromatin granules. The caeca are filled with a yellow fluid (presumably secreted by cells), which is coagulable by alcohol. The free border of the cells exhibit well-marked striation, which, as seen under the higher magnification, seem to be made up of closely set cilia-like filaments resting on a well-defined basal membrane.

The Salivary Glands are appendages of the fore-intestine. They are situated in the thorax and consist of a single gland on each side of the fore-intestine. Each gland is a hollow elongated tube lined by a single layer of cells, and passes at its anterior end into a thin

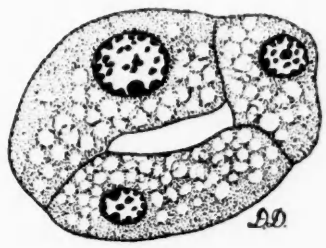

FIG. XIII. Transverse section of a salivary gland. (= × 400).

walled duct. The two ducts unite within the head close to the sub-oesophageal ganglion, and the common duct thus formed passes below the latter ganglion, and then bends upwards and outwards round the medial border of the imaginal bud of the labium, to open by a narrow oblique slit-like opening on a cone-like chitinous elevation (the hypopharynx), immediately dorsal to the labium. The direction

of the opening is outward towards the mouth cavity. Its lips possess strong fringes of hair which project over the labium. The duct expands into a slight bulbous enlargement just before its opening. The anterior lip of the external opening is continuous with the teeth on the dorsal margin of the labium. The posterior lip curves upwards and joins the chitin of the pharynx. The epithelial cells lining the glands are large and in the fresh condition

FIG. XIV. One of the salivary glands and part of its duct. (= × 90).

appear almost transparent. The cells are clearly defined and stain deeply with haematoxylin. The nuclei are extremely prominent and the chromatin is composed of coarse granules around the periphery of the nucleus. The cells sometimes bulge inwards to a certain extent into the lumen of the gland. The ducts are thin-walled and lined by a delicate intima with small definite nuclei.

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ON THE MANNER OF TRANSMISSION OF *MONIEZIA EXPANSA*, THE COMMON TAPEWORM OF THE SHEEP

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The life-history of the common tapeworm of sheep, *Moniezia expansa*, has remained one of the unsolved problems of helminthology. Many have worked upon it but though much of a negative character has been obtained little positive information is available. The literature has been reviewed by others from time to time but, in view of the impossibility of attempting to do justice to what has been recorded, and bearing in mind the fact that the purpose of this paper is to record the circumstances in which transmission was found to occur, reference will be made only to a paper describing a case in which transmission was successful. Daubney (1928) records that in an experiment carried out by him unmuzzled lambs allowed to graze over an infected paddock became heavily infested, whereas muzzled lambs of the same age, though running with them, did not. Daubney concludes that infestation is contracted by the mouth and that it is probable that some small invertebrate animal acts as intermediate host for the parasite. Mönnig (1929) has recently reviewed the literature and investigated possible means of transmission but with negative results.

In the investigations about to be described, lambs from a flock of ewes were removed at birth and then allowed to suckle the dams twice daily. At all times they were kept in concreted pens and when old enough were fed on wheaten chaff. Systematic faecal examinations were carried out weekly and at no time other than following a deliberate exposure to infection (mentioned later) has a lamb been found to be excreting ova of *Moniezia*. Some of the lambs, however, contracted a nematode infestation in spite of the precautions taken and they were then turned out in the paddock

in which pens A and B were later erected. When examined at the time of commencing the experiment these lambs were found to have contracted a tapeworm infestation from grazing in this paddock and they were therefore employed to super-infect the pens used.

In all cases the faeces of the sheep were examined for *Moniezia* ova weekly during the course of the experiment.

1930 INVESTIGATIONS

The observations made upon *Moniezia* in 1930 were incidental to the results of an investigation carried out by the writer and McGrath (1931) to gather information regarding the conditions requisite for the transmission of certain nematode parasites of sheep.

In these experiments it was found that *Moniezia* infestation was contracted by lambs grazing on areas which were quite different topographically. These areas are described in some detail in the publication referred to but the nature of each may be briefly stated as follows :—

PEN A. Low-lying grassed land with a permanent pool of water.

PEN B. Sloping grassed land without any area on which surface water might lie and with no swampy part.

PEN C. Like B.

PEN D. Part low-lying like A, remainder sloping like B.

PEN E. Bare ground. Actually a gravelled pen slightly sloping and devoid of vegetation.

Pens A and B were in the same paddock but pen E was some distance away and was one of the permanent pens used for holding stock. The sheep in all pens were supplied with water from the city supply and care was taken in the emptying of the troughs that such should be done outside the pens.

During the course of these experiments rain fell on many occasions and at one time pen A became flooded. Surface water from outside the enclosed area was diverted, however, from pens B and E.

That at least some part of the paddock contained the infestation was known at the commencement of these experiments in March, 1930, inasmuch as certain lambs of the same age but running in the

paddock for some time until five weeks before the experiment commenced were excreting *Moniezia* ova in the faeces. These lambs, like the others used in the experiment, were then about eight months old.

EXPERIMENT 1.

Two lambs (S. 572 and S. 567) were placed in Pen D on 13.iii.30 and grazed there continuously.

Result.—S. 572 showed ova of *Moniezia* on 57th day.
S. 567 showed ova of *Moniezia* on 64th day.

These lambs evidently picked up a residual infection in the pen.

EXPERIMENT 2.

Two lambs (S. 576 and S. 575) were placed in Pen A on 13.iii.30 and two lambs (one showing *Moniezia*) run with them, the animals being grazed there continuously.

Result.—S. 576 showed ova of *Moniezia* on 50th day.
S. 575 showed ova of *Moniezia* on 50th day.

Lamb 576 was killed on the 60th day and 66 cestodes were found. It was noted that all of these were mature or nearly so.

The infestation in this case might have been contracted from the residual infection in the pen; it might have come from ova voided by the affected lamb depastured with it.

EXPERIMENT 3.

Two lambs (S. 579 and S. 564) were placed in Pen B on 31.iii.30 and two lambs affected with *Moniezia* run with them, the animals being grazed there continuously.

Result.—S. 579 showed ova of *Moniezia* on 50th day.
S. 564 showed ova of *Moniezia* on 50th day.

S. 579 was killed on the 62nd day when 47 *Moniezia* were found.

Infestation in this case also might have come from the residual infection; it might have come from the infected lamb depastured with it.

EXPERIMENT 4.

Two lambs (S. 577 and S. 568) were placed in Pen C and two lambs (one affected with *Moniezia*) run with them.

This pen was similar to Pen B but the conditions of grazing were different, inasmuch as the experimental lambs S. 577 and S. 568 were allowed to graze only when the grass was dry.

Result.—S. 577 showed ova of *Moniezia* on 42nd day.
S. 568 showed ova of *Moniezia* on 49th day.

Infestation in this case also might have been contracted from the residual or the super-infection.

EXPERIMENT 5.

Two lambs (S. 581 and S. 574) were placed in Pen E on 13.iii.30 and two other lambs (one affected with *Moniezia*) run with them, the animals running there continuously.

Result.—S. 581 showed ova of *Moniezia* on 57th day.
S. 574 did not become infested (70 days).

Infestation in this case also might have come from the residual or the super-infection.

Experiment 6.

Two lambs (S. 570 and S. 566) were placed in a fly-proof concrete and brick loose-box and the water supply (in trough) deliberately fouled daily with faeces from sheep voiding ova of *Moniezia*. The water was approximately six inches deep and the water was never emptied. The floor was swept weekly.

Result.—Both remained free of infestation (90 days).

CONCLUSIONS FROM 1930 INVESTIGATIONS

Moniezia infestation was contracted by lambs running under the following conditions :—

(*a*) Not in contact with other affected sheep.

i. In pen D. Low-lying and sloping ground.

(*b*) In contact with sheep affected with *Moniezia*.

i. In pen A. Low-lying ground with pool.

ii. In pen B. Sloping ground.

iii. In pen C. Sloping ground grazed only when grass dry.

iv. In pen E. Gravelled pen.

As pens C and D were separated only by a netting fence it was not considered that contact had been sufficiently excluded though it was believed that the possibility of transmission through the fence was remote. (Experiments to be detailed later show that infestation is not transmitted by mere contact.)

The above experiment did show, however, that infection occurred even when the soil covering might vary considerably and particularly that the presence of surface water (a pool) or even of growing vegetation was not necessary. The gravelled pen, it may here be mentioned, was quite devoid of vegetation and the surrounding soil was kept chipped so that the sheep could get nothing by nibbling through the fence. There would be a little chaff and a few fallen leaves littering the ground at times, but the manure and littered chaff and leaves were swept up at intervals (not noted).

Experiment 6 showed that the *Moniezia* ova cannot develop to an infective stage in trough water.

In the positive experiments animals were first exposed to infection in March and from information gained in the 1931 experiments they evidently became infested immediately on exposure. The areas were therefore infective in that month.

In these also, young lambs specially raised cestode-free were employed and at the date of the first experiment (No. 7) would be about five months old. Repeated faecal examinations had been made and from the time of starting the 1931 experiments such were continued for all lambs at weekly intervals. At no time except after deliberate exposure to infection has a lamb shown an ovum of *Moniezia*. Except when depastured as shown below the lambs have been kept in concrete floored and walled pens, fed on wheaten chaff and have received drinking water from the city supply.

Pens A and B as used in 1930 were again employed and these pens, it may be noted, had been kept free of sheep for six months.

EXPERIMENT 7.

Two lambs (S. 700 and S. 709) were placed in Pen B. This pen was now well covered with grass, mostly rather harsh and drying off. It was arranged that they should be brought in if rain occurred, and as the aim was to give them a limited exposure, they were allowed to graze in the pen on only eleven days between 9th and 23rd January. At all other times they were kept in concreted pens.

Result.—Both lambs showed ova of *Moniezia* on 41st day after being placed in Pen B. (27 days after removal from pen.)

EXPERIMENT 8.

Lamb 699 was placed in Pen A together with two lambs which in 1930 had been infested with *Moniezia* but were not now (9.i.31) showing ova of that parasite in their faeces. S. 699 was allowed to graze for fourteen days and then removed to a concreted pen.

Result.—S. 699 showed ova of *Moniezia* on 45th day after being placed in Pen A. (31 days after removal from pen.) Killed and 23 *Moniezia* found. No immature forms.

EXPERIMENT 9.

Lamb 710 was drenched with an aqueous emulsion of fresh ripe proglottides from S. 699.

Result.—Remained negative (86 days).

EXPERIMENT 10.

Lamb 697 fed cut herbage (mostly grass) from Pen B. In this pen S. 700 and 709 (Experiment 7) had picked up infection though the pen had been empty for six months. These sheep had been allowed to graze there for only eleven days so that at the time the grass was cut the pen had been empty for a further three months. The grass was now about a foot high but a quantity was cut with a lawn mower and searched for invertebrate life. Any insects which could be found (and there were only a few, of varying types) were removed, together with faecal material and the grass then fed to S. 697. Such was repeated for seven days from 8th April, the lamb remaining in a concreted pen and thereafter being fed on wheaten chaff.

Result.—S. 697 showed ova of *Moniezia* on 48th day after first feed of grass from Pen B. (41 days after last feed of this grass.)

EXPERIMENT 11.

Lamb 712 grazed in Pen X. This pen was on grass land which had not been depastured by sheep for at least eight years. Faeces showing ova of *Moniezia* were strewn over the ground for twelve days from 25.ii.31. During the latter part of the time three inches of rain fell. Two days later (9.iii.31) S. 712 was placed in this pen and left there for four days. It was removed to a concreted pen and then on the 35th day from first exposure again placed in the pen for a further seven days (13.iv.31 to 19.iv.31).

Result.—Remained free (106 days from first and 61 days from second exposure).

It is to be noted that owing to being by itself this lamb did not settle down well and during the initial exposure grazed very little.

EXPERIMENT 12.

Lamb 701 was placed in Pen A on 9th April along with two other lambs, viz., S. 709 (affected in February but not now showing *Moniezia*) and S. 707 (injected subcutaneously with an emulsion of *Moniezia*).

Result.—S. 701 showed ova of *Moniezia* on 65th day.
S. 707 showed ova of *Moniezia* on 41st day.

EXPERIMENT 13.

Lamb 706 was placed in Pen A on 20th May, along with two other lambs, viz., S. 700 (affected in February but not now showing *Moniezia*) and S. 710 (drenched with emulsion of ripe segments of *Moniezia*).

Result.—S. 706 showed ova of *Moniezia* on 40th day.
S. 700 and S. 710 remained free and showed no *Moniezia* when killed on the 44th day.

CONCLUSIONS FROM 1931 INVESTIGATIONS

The first point definitely established in the 1931 experiments was that lambs depastured on infected ground contracted an infestation with *Moniezia* even though they were not in contact with infested sheep (experiments 7, 12, and 13). In the case of another experiment (8) other lambs, in contact with *Moniezia* infested lambs, likewise contracted an infestation with that parasite.

The second point is that a lamb, fed grass from the pen used in experiment 7, also contracted an infestation of *Moniezia* (experiment 10).

It was further shown (experiment 7) that grazing for a limited period during mid-summer and only when the herbage was dry, was followed by infestation. Also that the area (pen B) was infective six months after *Moniezia* infested sheep had grazed upon it (grazing, experiment 7) and again three months later was still infective (feeding experiment 10). In the case of pen A this pen was infective eleven months after *Moniezia* infested sheep had

been depastured three months (experiment 13). Ground evidently retains its infectivity for at least eleven months after contamination and it has been shown to be infective during the months of January, March, April, and May.

Infestation did not follow the ingestion of an emulsion of fresh proglottides containing viable ova (experiment 9), and another grazing experiment over an area whereon the only infection can have been from faeces intentionally strewn was also negative. This experiment may have been inconclusive for the reason that the sheep did not graze satisfactorily; it may have also been rendered abortive by reason of the very heavy fall of rain that occurred immediately following the spreading of infective faeces.

DISCUSSION

Before drawing the general conclusions from these two sets of experiments certain observations may be made.

It is evident that the nature of the ground over which the animals run plays no considerable part, i.e., neither surface water nor grass appear to be necessary and apparently bare soil may suffice. If an intermediate host is necessary, therefore, it is evidently not some aquatic invertebrate. Further, as infestation was contracted during mid-summer (January), autumn (March and April) and early winter (May), the invertebrate life on the surface of the soil may have varied quite considerably, seeing that at the first period the herbage was largely dried up though in the second and third it was green and actively growing. The fact that infestation was contracted from cut herbage carefully picked over by hand, and any invertebrate larger than a small ant excluded, would indicate that infestation is not through the swallowing of any other than a minute invertebrate.

Summing up the whole position, therefore, it would seem likely either (1) that no intermediate host is necessary, or (2) that the larvae pass through such host and assume an infective stage after leaving such host.

If no intermediate host is necessary the failure to infect by drenching living ova freshly secured might be explained on the assumption that it is necessary for such ova to undergo certain

development outside the body of the sheep before reaching an infective stage.

If the larvae actually undergo an essential part of their development in an intermediate host it would appear either (*a*) that they voluntarily leave that host and are then, in the free state, taken up by sheep, or (*b*) that after undergoing their essential development the host dies and disintegrates and the infective larvae are so liberated.

Though the pen E (gravel pen) experiment would suggest that encystment on herbage was not necessary, encystment on something (? scattered litter) was apparently possible in that pen for the common nematodes were transmitted also to these sheep.

An open mind is kept upon the question as to whether an intermediate host is necessary or not, but it is suggested that these experiments rather indicate that such is not necessary, that infection is direct but that certain extra-ovine development of the embryos is necessary.

CONCLUSIONS

1. The transmission of *Moniezia* is not directly from sheep to sheep by contact.

2. Infested sheep contaminate ground by their faeces and susceptible sheep become infested by later grazing such an area.

3. The nature of the soil covering plays no essential part. Neither permanent water nor grass appear to be necessary. If an encysted stage occurs such may apparently take place on litter (chaff and leaves).

4. Herbage cut from an infective area and fed may be followed by infestation.

5. As no recognisable invertebrate was present in such cut herbage it would appear that if the ingestion of the intermediate host is necessary such host must be minute in size.

6. An intermediate host may or may not be necessary. If it is, it is suggested that the larvae leave it and that the infective stage is a free stage, perhaps encysted. As direct feeding of fresh ova is not followed by infestation (Mönnig, 1929) it would appear that extra-ovine development of the ova is necessary before an infective

stage is reached, and it is thought probable that this may take place without the intervention of an intermediate host.

7. It has been shown that ground may be infective for at least eleven months after infested sheep have grazed upon it.

8. Infective ground has been shown to be infective in mid-summer, autumn and early winter.

(Other matters arising out of these experiments, particularly the life-cycle within the sheep and the development of immunity in sheep are being dealt with later in separate papers.)

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THE DEVELOPMENT IN SHEEP OF IMMUNITY TO *MONIEZIA EXPANSA*

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Though the development of an acquired immunity to certain nematodes and one trematode has been claimed and in certain few cases seems to be an established fact, such has not been demonstrated to occur in the case of cestodes. The literature on the question has recently been reviewed by Sandground (1929) who states that there is some evidence that a pre-existing infection may prevent the development of additional worms and that immunity to super-infection disappears after expulsion of the parasites. This has been claimed for *Taenia solium* by Brumpt and *Hymenolepis* by Joyeux.

The purpose of the present paper is to describe certain experiments which seem to offer no other explanation than that an actual active immunity is acquired by sheep naturally infested by *Moniezia expansa*, and that such persists after infestation has been lost.

PRIMARY OBSERVATIONS

In some experiments recorded elsewhere (Seddon and McGrath, 1931) certain lambs were raised free of all nematodes except *Strongyloides* sp. and later depastured on a section of grazing area which had been enclosed by a netting fence. With such lambs other lambs affected with *Moniezia expansa* were placed. After several weeks it was noted that the hitherto cestode-free lamb became infested with *Moniezia* (as shown by faecal examination and confirmed by post-mortem examinations) but the hitherto infested lambs similarly examined were now found to be free. Both types of lambs having been exposed simultaneously on the same infested area one would have expected the infested lambs to have

become re-infested and to have shown *Moniezia* at the same stage of development as the hitherto cestode-free lambs. As they did not it was at once evident that some immunological process was involved. It is, of course, common knowledge that lambs are much more frequently found infested by *Moniezia* than older sheep and it has been thought by some that such might be due to the development of an age-immunity. That this was clearly not the case in the experiments mentioned was shown by the fact that all the lambs were the same age (about eight months). Actually they were all from the same flock and those which formed the infested lambs for the above experiment were lambs which had developed a nematode infestation in spite of the precautions taken. They were, therefore, turned out to graze when they gathered a natural infestation with *Moniezia*.

In another paper (Seddon, 1931), we have mentioned (*a*) that lambs becoming infested with *Moniezia* harbour the parasite for a comparatively short time (approximately 65 to 70 days) and (*b*) that when an autopsy was made of lambs voiding ova of *Moniezia* only mature *Moniezia* and no immature forms were found. These observations likewise point to a limitation of susceptibility and the development of a resistance against at least super-infection.

To confirm the foregoing observations an additional series of experiments was carried out in 1931.

DETAILS OF 1931 INVESTIGATIONS

In another paper of this series we have recorded experiments in which it was shown that if one grazes a lamb, raised cestode-free on an area of ground over which *Moniezia* infested sheep have previously grazed (even though this may have been months before), that lamb may itself pick up an infestation with *Moniezia*. In other words transmission is by grazing over infested ground. Such an exposure was, therefore, the test of immunity in our experiment.

EXPERIMENT 1.

Lamb 709 allowed to graze over an infected area for eleven days between 9th and 23rd January and then replaced in concreted pen. Ova of *Moniezia* were first noted on the 41st day after exposure and continued to be voided in the faeces for 27 days. Further weekly faecal examinations until the 89th day (April 8th) were

all negative, and the lamb was then replaced on (and remained on) the infected area along with two lambs (S. 701 and S 707) raised cestode-free. (S. 707 had 33 days previously been given an injection of *Moniezia* subcutaneously—see experiment 5.) These lambs were of the same age—about nine months.

Weekly faecal examinations were continued and S. 707 was found to void *Moniezia* ova on the 41st day and S. 701 on the 65th day. Three days later post-mortem examination showed two adult *Moniezia* in S. 707; only a few ova of *Moniezia* in S. 701 and neither ova nor mature forms of *Moniezia* in S. 709. The fact that on post-mortem examination S. 701 did not show other than ova of *Moniezia* was disconcerting, especially in view of the fact that ova had first been detected in that lamb only three days before. In the absence of further knowledge we cannot well discuss this matter, and leave it for the present. As this was the initial specific test of immunity, using lambs of the same age, we are, however, in duty bound to report it. On the other hand, the fact of S. 707 showing *Moniezia*, and in a number such as one may expect in a lamb 68 days after exposure (actually losing infestation) shows at least that S. 709 had every opportunity of contracting infection but did not do so although continuously grazed on an infected area.

EXPERIMENT 2.

Lamb 700 was exposed to infection at the same time as S. 709, being penned with it, and like that animal, it first showed ova of *Moniezia* on the 41st day. The following two faecal examinations were negative after which ova in large numbers were again passed. These gradually diminished in number and ultimately disappeared entirely though odd ova were detected for such time that the total egg-excretion period was much longer than we have observed with any other sheep.

On 20th May (or 131 days since the animal was first exposed to infection and 26 days since ova had been seen in its faeces) S. 700 was again penned on the area where it had originally contracted infection. Two other lambs S. 710 (drenched with a suspension of *Moniezia*—experiment 4) and S. 706 (cestode-free control) were run with it and the animals were allowed to graze on the area continuously until the day they were killed.

Though faecal examinations were made weekly ova of *Moniezia* were not detected until the 40th day when they were found in the faeces of S. 706, the other lambs not voiding ova.

Four days later all three lambs were killed when 66 mature cestodes were found in S. 706 but none in S. 700 or S. 710.

This provided conclusive evidence of the immunity of lambs which have recovered from an earlier infestation with *Moniezia*.

EXPERIMENT 3.

Two lambs, S. 588 and S. 568 which had been originally infested in January and May, 1930, respectively and, as judged by faecal examinations, had become free of infestation, were placed on 9th January, 1931, in an infected grass pen. As a control of the infectivity of the pen, S. 699 (raised cestode-free) was placed with them for the first fourteen days and then removed to a concrete pen. S. 588 and S. 568 were left to graze in the pen continuously until 1st May.

S. 699 showed *Moniezia* ova on the 45th day after exposure to infection and when killed that day was found to harbour 23 mature *Moniezia*.

Though weekly faecal examinations were made of S. 588 and S. 568 for the whole five months they were exposed to infection, no ova of *Moniezia* were found. The immunity in these animals, therefore, appeared to last over a year.

EXPERIMENT 4.

This experiment was undertaken to ascertain if immunity developed following drenching with an emulsion of ripe proglottides of *Moniezia*. These were taken from S. 699 and three hours after collection 15 grms. of proglottides (but no heads) were ground in a mortar with sand and drenched to S. 710 (23rd February).

Repeated faecal examinations were negative and 86 days later (20th May) this animal was placed in an infective pen along with S. 706 (cestode-free control) and S. 700 (*Moniezia*-recovered). S. 706 showed ova on the 40th day after such exposure and when killed on the 44th day was found to harbour 66 *Moniezia*. Weekly faecal examinations of S. 710 were negative; when it was killed the same day as S. 706 it was found to be quite free of cestodes. (S. 700 likewise free—see experiment 2.)

Though a single experiment, the fact that drenching with an emulsion of freshly ground *Moniezia* was followed by immunity is significant.

EXPERIMENT 5.

This experiment was undertaken to ascertain if immunity developed following the subcutaneous injection of an emulsion of *Moniezia*. Ripe proglottides from S. 699 were used, being ground with sand and carbol saline to form a thick emulsion. This was allowed to stand at room temperature for eleven days and then 10 cc. injection subcutaneously into S. 707 (raised cestode-free), on 6th March.

On 8th April (or 33 days later) this animal was exposed to infection by grazing in a pen, with S. 709 (recovered) and S. 710 (control), and grazed there continuously.

S. 707 showed ova of *Moniezia* on the 41st day and when killed on the 68th day it was found to be still harbouring two mature *Moniezia*. Immunity did not, therefore, follow an inoculation with *Moniezia* emulsion.

DISCUSSION

The results of these several experiments are summarised in the table given (p. 435). Though in the case of experiment 3 the control animal was a year younger than the animals tested for immunity it will be noted that in the case of the other experiments the lambs involved were all of the same age. It thus appears that immunity in lambs under a year old definitely results from previous infestation but we are not prepared to deny that an immunity owing to age may develop in older animals. We have no evidence ourselves as to the finding of *Moniezia* in sheep of over eighteen months but it would appear likely that any immunity of sheep older than that may be due to an infestation during earlier life.

CONCLUSIONS

1. Lambs which have recovered from an infestation with *Moniezia* are immune to re-infection from grazing over infected ground.

2. Immunity may be conferred by drenching an emulsion prepared from freshly secured ripe proglottides. (Single experiment.)

3. Immunity was not found to follow the subcutaneous

inoculation of a similar emulsion which had been carbolised and stored for eleven days. (Single experiment.)

4. In those cases where re-exposure to infection was not followed by infestation the interval between last appearance of ova in the faeces and date of re-exposure has been 21 days, 26 days, 7 months, and 1 year, in the respective experiments. Where immunity followed drenching with fresh proglottides such drenching took place 86 days prior to exposure to re-infection.

It would appear, therefore, that immunity is developed certainly within six weeks of the primary infestation reaching maturity, and, from the fact that, apparently, super-infestation does not occur, at an even earlier period.

5. The facts suggest that immunity is local and concerns the intestinal mucous membrane.

TABLE.

Expt.	Sheep	History	Age of Sheep	Result of re-exposure
1 & 5	709	Previously infested	9 months	Remained free
	701	Not previously infested	9 months	Contracted *Moniezia* (Ova only seen)
	707	Subcutaneous inoculation	9 months	Contracted *Moniezia* (2 cestodes collected)
2 & 4	700	Previously infested	10 months	Remained free
	710	Drenched *Moniezia*	10 months	Remained free
	706	Not previously infested	10 months	Contracted *Moniezia* (66 cestodes collected)
3	588	Previously infested	17 months	Remained free
	568	Previously infested	17 months	Remained free
	699	Not previously infested	6 months	Contracted *Moniezia* (23 cestodes collected)

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ON THE LIFE OF *MONIEZIA EXPANSA* WITHIN THE SHEEP

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Prior to our recent experiments (1931) the only successful transmission of *M. expansa* seems to be that recorded by Daubney (1928) who, however, gives no information regarding the bionomics of the parasite.

In our experiments referred to the faeces of the experimental lambs were examined weekly for ova of *Moniezia* and as the period of exposure was in some cases quite limited it is possible to form some opinion of the period from infection to egg-laying. Also, as the lambs employed ultimately lost their infestation, certain information is available as to the length of life of the parasite within the sheep.

PERIOD FROM INFECTION TO APPEARANCE OF OVA IN FAECES

Information as to the time taken for the parasite to attain maturity may be gained from Table I.

In Table I information from the several experiments is detailed and from this it will be seen that, after exposure to infection, the shortest period in which ova of *Moniezia* were detected in the faeces was 40 days (S. 706). This does not necessarily represent the minimal period between infection and the presence of ova in faeces, as this sheep had not been examined during the previous two days. Further information in regard to this is obtainable from certain sheep which were exposed to infection for only a limited period. S. 699, it will be noted, had been *removed* from infection 31 days, S. 700 and S. 709 27 days, and S. 697 41 days prior to the appearance of ova. Other sheep grazed continuously showed ova in 41 days.

The minimal period before eggs may appear in the faeces cannot be less than 27 days and from S. 697 it would seem to be not less than 41 days. As, however, ova have been found in the faeces on the 40th day after exposure it would appear that the minimal period would be about 40 days. In other words, there is evidence that sheep in experiments 7 and 12 gathered infection the first day they were exposed ; also that in experiments 1 to 4 and 8 and 10 they

TABLE I.

Expt.	Sheep	Infested by	Days after first exposure					Cestodes found post-mortem
			Last negative examination	First positive examination	Last positive examination	First negative after infestation	Killed	
1	572	Continuous grazing	50	57	64	71	76	...
	567	Continuous grazing	57	64	71	78	...	...
2	576	Continuous grazing	42	50	57	...	60	66
	575	Continuous grazing	42	50	71	78	...	...
3	579	Continuous grazing	42	50	57	...	62	47
	564	Continuous grazing	42	50	71	78	...	...
4	577	Continuous grazing	35	42	69	70	...	...
	568	Continuous grazing	42	49	69	(Not examined further)		
5	581	Gravel pen Continuous exposure	50	57	88	...	88	1
7	700	Grazing 11 days	35	41	104	111	...	...
	709	Grazing 11 days	35	41	68	75	...	...
8	699	Grazing 14 days	42	45	45	45	45	23
10	697	Fed cut herbage for 7 days	41	48	77	...	...	...
12	701	Grazed continuously	63	65	68	...	68	...
	707	Grazed continuously	36	41	68	...	68	2
13	706	Grazed continuously	37	40	...	...	44	66

likewise gathered infection almost immediately they were exposed. The average period would appear to be 40 to about 50 days, i.e., six to seven weeks. In only two cases was this period greatly extended, viz., S. 567 and S. 701. In both these cases the ova found in faecal examinations were remarkably scanty and it is

possible that the sheep were actually voiding ova at an earlier date but that such were not detected.

The tentative conclusion reached therefore is that a period of approximately 40 to 50 days is taken between infection and the presence of ova in the faeces.

DURATION OF OVA PRODUCTION

If one accepts the presence of ova in the faeces as evidence of recent ova production by the cestode we find that sheep void ova for the periods shown in Table II.

TABLE II.

Sheep	Actual period	Possible period (Allowing for absence of faecal examination)
572	7	21 (Killed)
567	7	21
570	10	?
575	21	36
579	12	? (Killed)
564	21	36
577	27	35
568	20	?
581	31	? (Killed)
700	63	76
709	27	40
697	29	?
701	3	? (Killed)
707	27	? (Killed)

The striking fact emerges from these observations that sheep void *Moniezia* ova with the faeces for only a limited time, after which repeated faecal examinations fail to detect ova and this occurs even though the sheep be continuously grazed over infective

ground. The longest period for which sheep voided such ova was 63 days, but this was exceptional; further discussion of the case will be found later. In the case of those sheep which were not killed, ova were voided in the faeces for from 7 to 27 days. If we look at the possible period as shown in the table, i.e., period between the last negative faecal examination and the first negative faecal examination after infestation we find that, apart from S. 700, this varied from 21 to 40 days. From this it would seem that the period during which faeces may show ova is approximately 20 to 40 days. In those cases where ova were voided for over 27 days we find that the ova voided during the period in excess of 27 days were very scanty and possibly represented ova delayed somewhat in excretion after the parasite had died. Thus S. 581 killed on the 31st day after ova were first seen harboured only one tapeworm, though the previous faecal examinations would indicate that the infestation had earlier been much heavier. S. 697 at time of writing (29th day) shows only a very few ova indeed in the faeces. S. 700 is somewhat remarkable, inasmuch as the total period covering positive faecal examination covered 63 days. There were, however, two distinct periods of egg-laying with a fortnight in between during which no ova could be detected. It would possibly appear, therefore, that this animal had had two distinct infestations but the second closely following on the first. During the last 20 days, however, eggs were scanty indeed but nevertheless a few eggs could be detected on careful examination. (This animal will be discussed further in a paper concerning immunity to tapeworm infestation.)

The tentative conclusion reached, therefore, is that ova are voided in the faeces for an average period of 20 to 30 days though odd sheep may void eggs for a longer period. The limit, however, has been 63 days.

LENGTH OF PARASITIC LIFE IN THE SHEEP

From what has been said it will be seen that we have reason to believe that the life of the parasite in the intestine of the sheep is comparatively limited. Table III gives data bearing upon this point.

In the case of one sheep (S. 699) only was the period of exposure limited, but from the table it will be seen that the parasite must

have lived in the sheep for at least 31 days. From information obtained from other sheep as to the time between infestation and the appearance of ova in the faeces we consider cestodes must have been in S. 699 for at least 40 days. One notes also that in the case of all other sheep showing *Moniezia* on post-mortem examination that they had been exposed to infection for a period of over 40 days. One might possibly assume, therefore, that 40 days represents the minimal duration in the sheep, but, as this S. 699 on post-mortem examination showed 23 cestodes and these were by no means small, the indications are that the average minimal period is something in excess of 40 days.

TABLE III

Sheep	Number of *Moniezia* found on post-mortem	Period from first exposure to post-mortem	Period from last exposure to post-mortem
		days	days
581	1	88	0
572	0	76	0
707	2	68	0
701	0	68	0
579	47	62	0
576	66	60	0
699	23	45	31
706	66	44	0

As to the maximum period in the sheep we fortunately have sheep which were killed when showing only an occasional parasite or no parasites, although the previous infestation was at least 20 (and probably 50 to 60) S. 701 killed on the 68th day and still voiding odd ova showed no cestodes and S. 572 showed no cestodes though a week before (69 days) it was voiding ova. S. 707 showed only two cestodes when killed on the 68th day. These figures seem significant. On the other hand sheep killed on the 60th and 62nd days still showed considerable infestation, and S. 581 killed on the 88th day still showed an odd cestode. This sheep did not show ova in the faeces as early as other sheep and probably was not infested as soon after exposure.

These figures seem to suggest that the average duration of parasitic life in the sheep is from something over 60 or 62 to under 75 days, say 65 to 70 days.

CORRELATION OF THE PERIODS OF PARASITIC EXISTENCE IN THE SHEEP

One may now attempt to correlate the tentative conclusions in the three sections of this paper. We have, from information available, deduced that approximately 40 to 50 days is taken for the period between infection and egg-laying and that eggs are voided in numbers in the faeces for a period of approximately 20 to 30 days. Assuming that when the ova in the faeces have dropped to almost nil the parasite itself has died we get a total life of 60 to 80 days, the mean of which approximates closely the total length of life as deduced from the few post-mortem examinations we have been able to make.

CONCLUSION

It is considered that the duration of parasitic existence in the sheep is about 65 to 70 days, though individual parasites may live to about 75 days, that they take 40 to 50 days to reach maturity and that egg-laying continues for 20 to 30 days.

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OBSERVATIONS MADE BY DR. M. A. BARBER ON A MELANIC, COASTAL RACE OF *ANOPHELES COSTALIS*, GILES *(GAMBIAE)* IN SOUTHERN NIGERIA

BY

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INTRODUCTION

The present paper is mainly an account of some highly interesting investigations made by Dr. M. A. Barber, of the West African Yellow Fever Commission of the Rockefeller Foundation, in Southern Nigeria. Melanic specimens of *A. costalis* received from Mr. McHardy, Entomologist, Tanganyika, and collected in Dar-es-Salaam by Dr. J. M. Campbell are also recorded.

At the outset it should be stated that the term ' melanic ' is used only in the customary sense as implying specially dark forms of the species (see Imms, 1931). As pointed out by Imms, it is possible that the dark pigment may not be true melanin.

In 1903, Theobald described *A. costalis* var. *melas*, a ' very marked melanic variety ' of this species, which was collected from Bathurst, Gambia. The type, a female, differed from typical *costalis* notably in the following characters: the apical pale palpal band divided into two by an extra dark band; costa with only one pale interruption, except at the base; wing field darker; pale rings of tarsi greatly reduced; thorax dark brown to almost black, and abdomen deep black. Since this form was described a few other isolated specimens exhibiting marked melanism, especially of the integument and female palpi, have been collected; but apparently none have been found showing such a great degree of darkening of the costa and tarsi. The following are among the localities from which such specimens have been collected :—S. Nigeria: Lagos, Forcados, Port Harcourt, Ikang; Sierra Leone: Mando. The examples from Port Harcourt and Mando are in the collection of the British Museum

provisionally determined as var. *melas*. Most of these specimens seem to have been collected at or near the coast, but no special studies of the distribution or bionomics of these dark forms had been made before Dr. Barber began his investigations in Lagos recently. It should be noted here that the mere occurrence of an extra dark palpal band is a character which appears, apparently sporadically, in a small proportion of otherwise typical *costalis* from inland localities. The majority of these specimens are evidently individual variations, but in the type of *quadriannulatus* the extra dark band is very extensive so that the extreme tip only of the last segment bears pale scales; such forms may represent a distinct variety. It should also be noted that in caught females of typical *costalis*, that have recently fed, the integument of the thorax and abdomen usually appears much darker than usual.

Barber and Olinger (1931) briefly summarise the results of observations and experiments on a dark race of *A. costalis* associated with coastal waters near Lagos and Dr. Barber has very kindly handed over to me for study, samples of the material collected and bred out during the course of these investigations.

COLLECTIONS MADE IN NATURE BY DR. BARBER

From Apapa, near Lagos, 12 October, 1930: ♂ and ♀, markedly melanic, both with very dark blackish integument; ♀ palpi with the extra dark band well developed. The dark scales of the wings, legs, palps, etc., were markedly blackish but, as this character seems to vary a good deal among typical *costalis*, it will not be referred to again. Larval pelts of these specimens with all chitinous structures, head capsule, tergal plates, bristles, palmate bristles, pecten, etc., very much darker, and fronto-clypeal markings more extensive than in normal *costalis* larvae. The pecten also showed differences from that of typical *costalis*. In the latter there is usually a very definite alternation between the long teeth and groups of about two to five shorter teeth which are more or less equal in length. In the brackish water larvae, however, there was a much less definite alternation, all or some of the shorter teeth approaching the longer ones in length. Two preserved larvae from the same collection showed most of these characters and, in addition, the

dorsal surface was darkly pigmented ; the pecten teeth were rather broken. Another larva from the same locality taken with several *obscurus*, mostly from brackish water, December, 1930, resembled these two and the pecten showed a much less regular arrangement of long teeth and groups of short ones than in normal *costalis*. The pelt of a larva taken in salt water from this locality, January, 1930, agreed with those described above and the associated pupal pelt was much darker in colour than in typical *costalis*. The pelts of five out of six pupae taken in salt water, Apapa, also showed markedly dark colouration.

Lot V, reared from larvae taken in coastal swamp, of which the water was 46 per cent. sea water at the time of collection : 1 ♂, 2 ♀♀, markedly melanic. In all these the integument was blackish ; in 1 ♀ the palpi showed the extra dark band, in the other these were normal. There was a distinct tendency to reduction of the tarsal rings.

Lots A and C, reared from larvae taken in fairly brackish water in coastal swamp, Lagos : 5 ♂♂, 4 ♀♀, distinctly melanic ; all with integument, black or blackish brown. Palps of 1 ♀ with ochraceous scales tending to form a sub-apical dark band, those of other ♀♀ normally three-banded.

Lots Q, R and S. Larvae and imagos from a locality near the sea, but containing *fresh water* at the time of collection.

R : 1 ♀, melanic, with blackish integument, 1 palp definitely 4-banded and the other with a tendency to form an extra dark band.

Q : 13 ♂♂, 12 ♀♀, tending to show melanism. Integument distinctly darker than in typical *costalis* and, in some cases, approaching the condition of specimens A and C ; ♀ palpi all normally 3-banded.

S : 25 larvae. Much paler than Apapa larvae. General colouration and that of the chitinous structures very little darker than in typical *costalis*. Pecten practically normal.

Lots T and U, from a much shaded pool near the sea, fresh water.

T : 3 larvae, 2 practically normal, 1 showing some degree of pigmentation of the palmate bristles.

U : 3 ♂♂, 6 ♀♀. Integument of most specimens very little darker than in typical *costalis*, but in one or two a rather marked darkening, not so great as in A and C ; ♀ palpi all normal.

M, N, O, P. Several ♂♂ and ♀♀ bred out from fresh water away from the coast in S. Nigeria. These specimens nearly all showed a normally light or moderately light brownish integument, but in one or two it was greyish in colouration ; ♀ palpi all normal.

These records show that the melanic forms of *costalis* collected by Dr. Barber in S. Nigeria, were all associated with coastal waters, and with one exception (R), those exhibiting a marked degree of melanism developed in brackish or saline water.

Dr. Barber has proved that *this form is an efficient vector of malaria parasites.*

The association between these dark forms and brackish water was further brought out by the results of experiments carried out by Dr. Barber, which are described below from notes kindly supplied to the writer.

DR. BARBER'S EXPERIMENTS ON THE DEVELOPMENT OF EGGS OF *COSTALIS* OF DIFFERENT ORIGIN IN FRESH AND BRACKISH WATER ; CARRIED OUT IN LAGOS (EXCEPT WHERE OTHERWISE STATED), 1931

Experiment I.

Material used. Eggs of *A. costalis* collected at Ibadan, 6 January. 'Ibadan is situated about 100 miles north of Lagos and probably no salt or brackish water occurs within 20 miles of the city.' Eggs laid on the night of January 7-8.

Conditions. The eggs were divided into three batches, some hundreds to each, and placed in containers in the laboratory with different types of water, as follows :—

Lots 1 and 2. *Controls.* Tap water ; crushed water plants as food.

Lot 3. Brackish water obtained from Apapa jungle where the melanic form of *costalis* was abundant. Plentiful food supplied.

Results. By January 9, eggs had hatched in all the lots. The next day the larvae in the brackish water were abnormally slender and two days later were all dead or moribund ; they were all dead by 13 January. The controls which had been developing normally were about half grown by this time, and by the 20 January had given rise to six or seven ♀♀.

EXPERIMENT II.

Material used. 'Eggs of *A. costalis* collected in native houses, Apapa, situated about half a mile from Apapa jungle. These *costalis* presumably consisted of, or contained, the (?) variety capable of breeding in brackish water. Eggs were laid on the night of January 15-16.'

Conditions. The eggs were divided into four batches and placed in containers in the laboratory with different types of water as follows :—

Lot 1. 'The same water and same container as that used for Experiment I, i.e., brackish water from Apapa jungle, the water may have fallen $\frac{1}{4}$ in. to $\frac{1}{2}$ in. in container and thus become slightly more brackish.'

Lot 2. 'Brackish water collected from Apapa jungle, January 20. Analysis made January 20, by Water Examiner for Government Analyst, Lagos—pH. 7·0 ; Chlorides expressed as Cl., 863 parts per million. This chloride content corresponds to about 46 per cent. sea water. Therefore of similar composition to Lagos lagoon water.'

Lot 3 *Controls.* Fresh tap water.

Lot 4. *Controls.* Lake water.

Results. Lot 1 (brackish water in which Ibadan larvae had died). In ten days the larvae were nearly full-grown and on 26 January one pupa appeared 'which later emerged as a dark *costalis.*' Lot 2. By 27 January, twelve pupae had appeared.

Controls. Pupae appeared on January 26 and 27.

From the results of this experiment, Dr. Barber concluded :—'Thus this type of *costalis* flourished in both fresh and brackish water.' Specimens which hatched in the course of these experiments are described below.

EXPERIMENT III.

Material used. Eggs of *costalis* from Ibadan laid on night of January 22-23.

Conditions. The eggs were divided into two batches and kept in containers in the laboratory as follows :—

Lot 1. *Controls.* Fresh water, the same as used in experiment II, 3.

Lot 2. Some of the brackish water from Apapa used in

experiment II, 2 (see above); this water may have evaporated slightly, but was *diluted* with an equal volume of water from the lake. It was still brackish to the taste.

Results. By January 24, eggs had hatched in both lots. On the third day those in lot B were all dead and very small, but the controls appeared to be developing normally. The experiment was discontinued at this stage.

EXPERIMENT IV.

Material used. 'Eggs from *Anopheles* spp. (almost certainly a mixture of *costalis* and *funestus*) sent by Dr. Drysdale Anderson, from the inland locality Abeokuta. It is doubtful whether brackish water exists in the vicinity of this place or not.'

Conditions. The eggs were divided into two batches and placed, on January 19, in containers in the laboratory with water of different types as follows:—

Lot 1. Brackish water, some of that used in experiment II, 2 (analysis given above). Water not diluted.

Lot 2. *Controls.* Fresh water from the lake; larvae transported to Liberia at end of January.

Results. After three days there was no sign of growth of larvae in Lot 1, which were all dead. The control larvae developed normally, giving rise in time to adults of *costalis* and *funestus*, in spite of transportation to Liberia. It was noted that the larvae of *funestus* took longer to develop than those of *costalis*.

DESCRIPTION OF EXAMPLES REARED OUT BY DR. BARBER IN EXPERIMENT II

Lots 1 and 2, developed in brackish water: 8♂♂, 14♀♀. Integument of thorax and abdomen markedly blackish in all specimens. Three ♀♀ with a well-developed extra dark palpal band and this distinctly indicated in two others; the tarsal rings tending to be narrow in about half the specimens.

Lots 3 and 4, developed in fresh water. 5♂♂, 9♀♀. Integument of most of the specimens showing practically normal pale brownish colouration but, in three, distinctly darker and greyer than normal, though paler than in Lots 1 and 2. In one ♀ the palps showed a distinct indication of an extra dark band on one palp, and in another

there was a very slight suggestion of this on both palps (pale ochraceous scales present but palps somewhat rubbed).

The results of this experiment strongly indicate that the form or race of *costalis* which is capable of developing in brackish water, becomes much blacker in integument and tends to show the extra dark palpal band in a higher percentage of females when reared in brackish water than in fresh water.

DISCUSSION

From the above records it seems clear that Dr. Barber has demonstrated the existence of a race of *costalis* with marked melanic tendencies, which breeds in the coastal swamps near Lagos, and which differs from forms of *costalis* found inland in Southern Nigeria, in that the larvae are capable of developing in brackish water. It would also appear that the colouration characters of this race are highly unstable, the full degree of melanism being only developed when the larvae are reared in brackish water, or at least, in water from the coastal swamps. Dr. Barber notes that even in the season when the water of the coastal swamps becomes fresh, these waters differ from ordinary fresh water, the mud and underlying materials being peculiar. In this connection it is interesting to record two specimens recently received from Mr. J. S. McHardy. The specimens, a male and female, were reared from larvae collected by Dr. J. M. Campbell, in ' dark brown rain water pools in a swamp, Dar-es-Salaam (water, however, was not acid although it looked peaty enough, pH. 7·8).' As Mr. McHardy notes, these specimens both have the integument unusually dark and the female palpi show a well-marked extra dark band, but the tarsal rings are broader and more distinct than in Dr. Barber's melanic forms. It is possible that this represents a melanic race which has arisen on the East Coast of Africa independently from that found in Southern Nigeria.

The question arises whether Dr. Barber's coastal race is the same as Theobald's ' *costalis* var. *melas* ' or not, and reference must be made to Christophers' (1924) conclusion that ' there is no reason to suppose that this constitutes a true local variety. The fourth palpal band and bridging of the pale costal spots are among the ordinary manifestations of melanism in *Anopheles*.' From

Dr. Barber's observations, however, it is seen that the general blackening of the integument is the most constant characteristic of the melanic forms found in Lagos. This is also a feature of Theobald's type specimen, but the question whether this represents an extreme form of the same race or belongs to another which has developed independently could, apparently, be settled by examination of a series of specimens collected from the saline waters near Bathurst. Dr. Edwards, who kindly re-examined the type, noted that the front tarsi were entirely unbanded and, although Dr. Barber's dark specimens exhibited a tendency to reduction of the tarsal markings, none showed this in so great a degree as in the type of *melas*, nor was there any marked melanism of the costa. In other respects, however, the darkest Lagos forms agree pretty well with the type of *melas*. In this connection it may be mentioned that amongst a collection of material in a poor state of preservation, I have seen from Bathurst, was a female in which the costa was distinctly melanic but the palps practically normal. It would appear, therefore, that the Gambia race is possibly subject to much variation.

Dr. Barber's observations raise the further question whether all the *costalis* which breed in brackish coastal waters in West Africa belong to a race or races which are distinct from those occurring inland. Dutton (1903) records the finding of larvae of *A. costalis* in brackish and salt water at Bathurst, and Giles (1905) found the species together with *Culex thalassius* breeding in brackish puddles about the Lagoons in Sekondi, the water in one of them containing 2,100 parts of chlorine per 100,000. In Accra, Ingram and Macfie (1917) record the finding of larvae of both these species in a pool flooded by the sea at high tide. Dutton found by experiment that eggs of *costalis* were laid in a tub containing ' sea water, taken as the tide was coming in,' and gave rise in eleven days or less, to adult mosquitos. He also found that larvae of this species from various sources remained alive in water containing up to 75 per cent. sea water, but that many of them died in higher concentrations. No mention is made of specially dark colouration in these specimens, but it is quite possible that this character was thought at that time to be merely due to individual variation. Balfour (1921) mentions no abnormality in the *costalis* he found breeding in brackish pools formed by seepage in North Khartoum.

It is well known that in other species such as *A. rossi* Giles (*subpictus*) and *A. ludlowi* Theo., fresh and salt water races are distinguished, and in some cases, these are said to differ in colouration or structure. Walch and Soesilo (1929) have recently described differences in the pectens of certain of these races in the Dutch East Indies. They show that in this region, in the salt water form of *A. rossi*, this differs from that of the fresh water form in having the shorter teeth approaching the longer teeth in length, the aspect of the pecten being described as irregular in the former. In the Philippines, however, according to Urbino (1930), the pectens of both salt and fresh water *rossi* are similar and have the teeth very unequal, while salt water *ludlowi* is distinguished from its fresh water form and from *rossi* in having the teeth all about equally long and uniform. This author also describes differences in colour markings and structure between the adults of the fresh and salt water forms of those species, and it is interesting to note that ' hypermelanism ' (the occurrence of an extra dark palpal band) occurs very frequently in *fresh* water *ludlowi*. The characteristics of the brackish or salt water forms of these three species of the Pseudomyzomyia group are, therefore, somewhat variable but it would appear that, when the pecten differs from that of the fresh water forms, it is in the direction of lengthening of some or all of the shorter teeth. Melanism, on the other hand, is by no means a constant feature of the adults of the salt water forms, however, the reverse being the case with *ludlowi*. The colouration difference between typical *A. maculipennis* and the salt water variety, var *atroparvus* van Theil, appears to be somewhat analagous to that between *A costalis* and the salt water forms here described. Van Theil (1929) notes that the colouration of his variety is more greyish than that of the type and that the spots and costal border of the wings are darker in the variety than in the type.

Even in *costalis*, melanism may be quite absent in specimens bred in saline water. Dr. Edwards kindly showed the writer a series of specimens of this species collected by Dr. J. T. Bradley, in 1930, on the island of Aldabra, where practically all the available water was saline. These specimens were quite normal in colouration, although they must, apparently, belong to a race that is biologically distinct from that found inland in Southern Nigeria.

Thus Dr. Barber's race possesses the peculiarity of being able

to develop in brackish water and of exhibiting a marked degree of melanism when bred in such water. This latter characteristic is interesting in view of the investigations undertaken into the effect of various chemical factors in the melanisation of certain Lepidoptera, summarised by Imms (1931). Although there are not sufficient data to discuss the actual factors concerned in the present case, the composition of the water appears to have some influence, either directly or, as suggested by Dr. M. G. Blacklock, through its effect on the food supply, in bringing about the very marked melanism of this coastal race of *costalis*.

CONCLUSIONS

It seems justifiable to draw the following conclusions from the results of Dr. Barber's investigations.

1. A race of *costalis* capable of developing in normal time in water of salinity at least equal to 46 per cent. sea water, breeds in the coastal swamps near Lagos.

2. The race is melanic, especially in the colouration of the integument of the adults, and pigmentation of the body and chitinous parts of the larvae, but the melanism attains its maximum development in specimens bred in coastal waters, especially when these are brackish.

3. Larvae hatched from eggs of *costalis* from certain inland localities in Southern Nigeria, failed to develop in brackish water, in which those of the coastal race developed successfully.

4. Larvae hatching from eggs of *funestus* originating from Abeokuta failed to develop in brackish water (slightly more than 46 per cent. sea water).

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ON TWO NEW SPECIES OF NEMATODES FROM *PELECANUS ONOCROTALUS*

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During 1930, four specimens of *Pelecanus onocrotalus* were dissected and examined for helminth infection. One of these animals was caught in Lake Manzallah, and the other three died in the Cairo Zoological Garden, and were also of Egyptian origin. Two of the birds contained, besides other helminths, the two species of Acuarinae described in this paper as new to science.

SYNHIMANTUS SIRRY n. sp.

Host : *Pelecanus onocrotalus* (White Pelican).

Locality : Lake Manzallah, Egypt.

Habitat : Gizzard.

Morphology : The worms are slender and tapering at both ends. The female is about 9 mm. in length and 0·3 mm. in maximum diameter. The male is shorter and more slender. It measures 6·8 mm. in length and 0·16 mm. in maximum diameter. The cuticle is thick and finely striated transversally. The head is ornamented with two cordons lying laterally. Each cordon has one recurrent loop. The cordons project slightly from the surface of the body, especially at their distal margin. The cordons run a sinuous course and reach as far distally as 300μ from the head in the male, and 320μ in the female. The recurrent loop has also a sinuous course and reaches as far as 75μ from the head in the male and 80μ in the female.

The mouth is simple and is provided with two lips, each surmounted by one projecting spine. The lumen of the mouth is surrounded by a tubular cuticular wall.

The alimentary canal begins with a long, narrow, sinuous vestibule measuring 290μ in the male and 320μ in the female. This leads into a muscular oesophagus having also a sinuous course and opening after

curving laterally into the chyle-intestine. It has an almond-shaped muscular bulb. The oesophagus is 400μ long in the male and 420μ in the female. The chyle-intestine is a simple straight tube ending in an anus lying 150μ from the tip of the tail of the female. The female tail is bent always dorsally. In the male the opening of the cloaca lies at 160μ from the tip of the tail. The cervical papillae are short, thick, and tricuspid. They lie at a distance of 370μ from the head in the male, and of 400μ in the female.

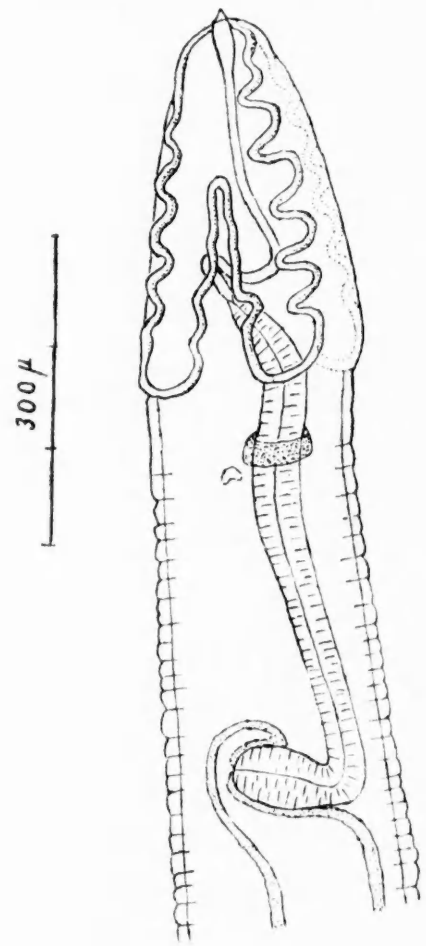

FIG. 1. Cephalic end of *S. sirry* n. sp.

The nerve ring is seen with difficulty. It surrounds the cephalic end of the muscular oesophagus a little cephalad of the level of the cervical papillae.

The excretory pore lies a little caudad of the level of the nerve ring.

The Genital organs: The male tail is coiled upon itself to more than a complete circle. There are two cuticular alae of small

breadth. The number of peri-anal papillae is nine, of which four are pre-anal and five post-anal. In addition, there are four small papillae near the tip of the tail in a median position.

The two spicules are distinctly unequal in length and dissimilar in shape. The right spicule is long and thin, being 410μ in length. Its tip is provided with alae. The left spicule is very short and massive. It is 130μ in length and is arched into a boat-shaped mass. The tail of the male is 160μ in length. It ends bluntly.

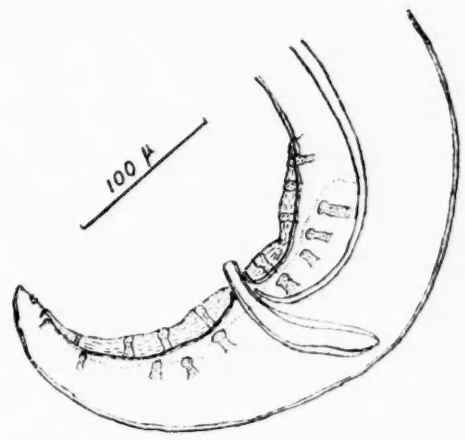

FIG. 2. Lateral view of the tail of *S. sirry* n. sp.

The female genital organs consist of two ovaries and two uteri. The vagina opens into a vulva situated nearer the tail than the cephalic end at practically 4/7 the body length from the head end. The ova are oval in shape and are provided with a thick shell. The ovum measures 37μ by 25μ.

The type of this species is deposited in the Parasitological collection of the Faculty of Medicine, Cairo, Egypt. Coll. No. 2133.

The sub-family Acuarinae was created by Raillet, Henry and Sisoff, in 1912, to include Spiruroidea provided with cutaneous cordons at the anterior end. The type genus Acuria was tentatively subdivided into five subgenera of which Synhimantus was meant to include those species with cordons recurrent and anastomosing on each lateral surface. Twelve species in birds are included in this genus, of which one, *S. raillieti* Skrjabin, 1924, is from a pelican species. Only the female was described. It is said, according to Cram, to have seven cordons on the head. This species was named *S. sirry*, in recognition of the facilities provided by H. E. Hussein

Sirry Bey, Under-Secretary of State for the Ministry of Public Works, Egypt, sanctioning that all animals dying in the Giza Zoological Gardens shall be submitted to the author for the collection of parasitological material.

COSMOCEPHALUS FARIDI n. sp.

Host : *Pelecanus onocrotalus* (White Pelican).

Locality : Lake Manzallah, Egypt.

Habitat : Gizzard.

Morphology : The worms are very thin and elongated. Only two males and one immature female were found. The males were longer than the female, averaging 7·6 mm. in length, while the single female was 5·7 mm. in length. They were found in common with the *Synhimantus sirry* in the same animal.

The cuticle is fairly thick and finely striated transversally. The head is ornamented with two cordons lying laterally. Each cordon at its cephalic commencement forms a posteriorly directed loop, one on either side. The cordon has a sinuous course reaching posteriorly as far as 400μ from the cephalic end in the male and 300μ in the female. The cordon is recurrent and anastomosing. The recurrent loop reaches as far as 110μ from the cephalic end in the case of the male, and 100μ in the case of the female. The cordons have no scalloped edges and project slightly from the surface of the body. The mouth is simple and is provided with two simple lips. The alimentary canal begins with a long, narrow vestibule, often bending to one side in its course, forming a loop. The vestibule ends in the oesophagus at the level of the caudal end of the cordons.

The oesophagus is very long and runs a sinuous course opening into the chyle-intestine. The bulb of the oesophagus is globular, opening into the intestine with a lateral bend. The oesophagus measures 930μ in the male, and 680μ in the female.

The chyle-intestine is a simple straight tube ending in an anus, lying 180μ from the tip of the tail in the female. The female tail is straight.

The male cloaca lies at 270μ from the tip of the tail.

The cervical papillae are simple, short and thick They lie at the level of the cephalic end of the oesophagus immediately posterior

to the cordons. The nerve-ring surrounds the muscular oesophagus, a short distance caudad of its commencement.

The excretory pore lies caudad of the level of the cervical papillae.

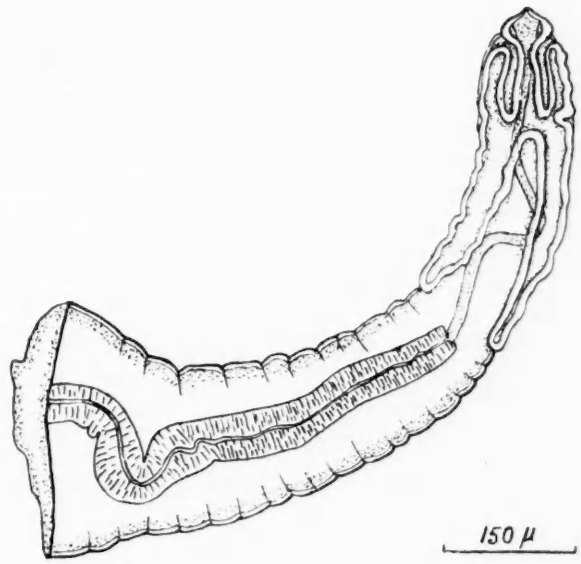

FIG. 3. Cephalic end of *C. faridi* n.sp.

The Genital organs: The male tail is coiled upon itself into a half-circle. There are two short cuticular alae. The number of peri-anal papillae is nine pairs, of which four pairs are pre-anal and five pairs post-anal. The papillae lie close together, except the caudal pair, which lies isolated near the tip of the tail.

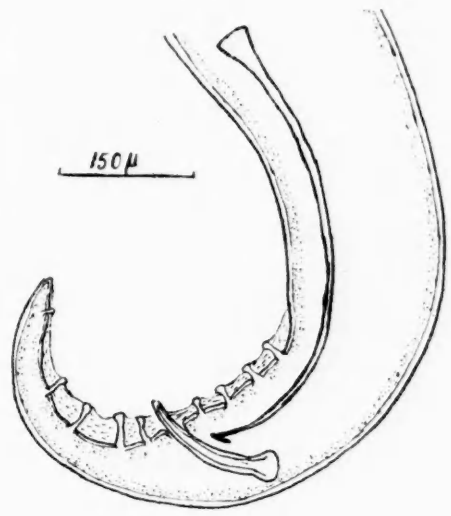

FIG. 4. Tail end of male *C. faridi* n.sp.

The two spicules are distinctly unequal in length and dissimilar in shape. The long spicule is thin and has a recurved tip resembling a fishing hook. It is 540μ in length. The short spicule lies transversally in the body and is thick. It is 160μ in length.

The female genital organs consist of two ovaries and two uteri. The vagina opens into a vulva situated about the middle of the body, nearer the cephalic than the caudal end. The type of this species is deposited in the parasitological collection of the Faculty of Medicine, Cairo, Egypt. Coll. No. 2127.

Discussion

The genus *Cosmocephalus* Molin, 1858, contains five species :—

1. *C. Diesingii* Molin, 1858, from *Larus capistranus.*
2. *C. aduncus* Creplin, 1896, from *Colymbus* sp.
3. *C. asturis* Yorke and Maplestone, 1926, from *Astur tachino.*
4. *C. obvelatus* Creplin, 1825, from *Larus maximus.*
5. *C. faridi* Khalil, 1931, from *Pelecanus onocrotalus.*

Judging from the drawing of *C. asturis* by Yorke and Maplestone, it is apparent that this species does not belong to the genus *Cosmocephalus*, but rather to the genus *Synhimantus*. The description of the other species is not detailed and needs revision.

This species was named *Cosmocephalus faridi*, in recognition of the facilities provided by Dr. Ahmed Bey Farid, Director of the Veterinary service of Egypt, including the Giza Zoological Gardens.

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STUDIES ON MALARIA IN SOUTHERN NIGERIA*

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WITH AN APPENDIX BY

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I. THE MALARIA PARASITE INDEX OF CERTAIN AGE GROUPS AMONG AFRICANS

In this part of the work our aim was primarily to compare different groups of a population and, in order to obtain fairly uniform results, all blood specimens were examined by one person (M. A. Barber). We used thick films and the Giemsa stain in all cases, and, as a routine, examined 150 microscopic fields before declaring a specimen negative. For comparing the degree of parasite infestation of different cases we adopted a standard field of approximately uniform thickness.

The persons examined consisted of infants and their mothers coming to the Health Offices of Lagos and Ebute Metta for vaccination, school children, children from police barracks, labourers, laboratory assistants, and people from miscellaneous sources, all residing within a radius of twenty miles from Ebute Metta. No hospital cases were included ; there was no selection of persons ill of malaria or of any other disease, and the series represents fairly well the malaria parasite status of persons in ordinary health.

Certain school children, a few groups of adults, and about 1 per

* The studies and observations on which this paper is based were conducted with the support and under the auspices of the International Health Division of the Rockefeller Foundation.

cent. of infants appearing for vaccination were re-examined. The results of these re-examinations were included in our series if the second or later examination was done a month or more after the first. The inclusion of these re-examinations introduces no appreciable error since they comprise an inconsiderable percentage of the total, and there was no selection of groups or individuals likely to have a very high or low rate.

The ages of infants coming to health offices for vaccination were accurately known since the date of their birth is on record. Those of older persons could be only approximately determined; but, although the exact age of an individual might be uncertain, the limits of a large age group should be reasonably precise.

The results of all examinations appear in Table I, and are rearranged in Table II, where certain age groups are combined.

TABLE I.

Parasite indices of certain age groups of Africans.

Age groups (inclusive)	Among all cases				Among malaria-positive cases				
	Number examined	Percentage positive for			Number of malaria positives	Percentage with			
		Malaria	Filaria	Marked anaemia		Heavier infestation*	Quartan	Crescents total	Crescents heavier infestation†
1 month	16	25·0	0·0	25·0	4	50·0	25·0	25·0	0·0
3 months	1,200	22·4	0·0	2·8	269	57·6	5·5	34·9	8·5
4 months	205	29·7	0·0	5·8	61	67·2	3·2	45·9	8·2
5–11 months	243	57·6	0·0	6·2	140	70·0	12·8	29·3	2·9
1 year	80	90·0	0·0	3·8	72	76·4	25·0	36·1	4·2
2 years	78	93·6	0·0	2·6	73	78·1	25·6	24·7	1·4
3 years	110	98·2	0·0	2·8	108	76·9	30·5	23·1	1·9
4 years	156	93·6	0·0	3·8	146	72·6	34·2	24·6	0·7
5–8 years	1,104	93·7	0·5	0·5	1,034	58·4	29·1	10·8	0·3
9–12 years	919	86·7	0·9	0·0	797	32·0	22·4	7·4	0·0
13–15 years	638	76·2	1·3	0·3	486	23·0	23·6	7·5	0·0
16 years and over	2,139	48·6	3·7	0·3	1,039	9·3	19·8	7·7	0·0
All ages ...	6,838	61·4	1·5	1·3	4,229	39·4	22·6	13·3	1·2

* One or more parasites per thick-film field.
† One or more crescents per 100 leucocytes. Usually 500 or more leucocytes were counted.

TABLE II.

Parasite indices of Africans. Certain age groups combined.

Age groups (inclusive)	Among all cases				Number of malaria positives	Among malaria-positive cases			
	Number examined	Percentage positive for				Percentage with			
		Malaria	Filaria	Marked anaemia		Heavier infestation*	Quartan	Crescents total	Crescents heavier infestation†
1–11 months	1,664	28·5	0·0	3·7	474	62·4	7·6	34·4	6·7
1–4 years	424	94·1	0·0	3·3	399	75·4	30·0	26·2	7·5
5–8 years	1,104	93·7	0·5	0·5	1,034	58·4	29·0	10·8	0·3
9–12 years	919	86·7	0·9	0·0	797	32·0	22·4	7·4	0·1
13–15 years	636	76·2	1·3	0·3	486	23·0	23·6	7·5	0·0
16 years and over	2,139	48·6	3·7	0·3	1,039	9·3	19·8	7·7	0·0
All ages ...	6,688	61·4	1·5	1·3	4,229	39·4	22·6	13·3	1·2

* One or more parasites per thick-film field.
† One or more crescents per 100 leucocytes.

Comment on Tables I and II.—1. The malaria parasite index, relatively low among infants 1 to 4 months of age, rises rapidly between 5 and 11 months, reaches a maximum in the age group 1 to 4 years, falls slowly until 16 years is reached, then drops to a comparatively low level in the age group 16 years and over. It is obvious that the total percentage positive, 61·4, is far below that of the population in general, for our material included disproportionately large numbers in the age groups which show the lowest index. The same caution applies to the totals in all columns of the tables.

2. Filaria appears in the age group 5 to 8 years, then steadily increases to age 16 years and over. No special examination was made for filaria, and as a rule the positives include only cases in which filaria was detected in the routine examination for malaria parasites. In one group of forty labourers we found 25 per cent. infected with filaria.

3. Anaemia was much commoner among infants and very young children than among older persons. We noted only such cases as were so marked that the condition appeared plainly in the thick film. The percentage among adults is increased by cases found among

mothers of young infants. Anaemia was usually associated with malaria parasites except among these adults.

4. Quartan malaria exhibits a low index among infants, rises to a maximum in age groups 1 year to 8 years, then declines but remains high in adults. We noted as quartan any case in which quartan parasites were detected. The majority of such cases show malignant tertian parasites as well. Apparently infants are as a rule first infected with the malignant tertian parasite, the commonest parasite in Southern Nigeria. They may later acquire quartan parasites, which, being a resistant species, tends to persist for years. We once found quartan parasites in an infant 2 months old.

5. The incidence of total crescents is high among infants and children of 1 to 8 years. It then drops to a constant in age group 9 to 12 years. 'Crescents total' includes any case in which one or more crescents were found. We find the general crescent index in Southern Nigeria about the same as that among West Indian negroes in Central America.

6. The index of heavier crescent carriers (those having one or more crescents per 100 leucocytes) is high among infants and young children, low at 4 years, then rapidly falls to nil. (See Section IV for a discussion of the relation of the crescent index to the infection of *A. costalis*.)

The re-examination of certain groups done after an interval of one month or more has afforded us some interesting results. In a group of 116 school children from St. Paul's Breadfruit School, Lagos, a single re-examination raised the percentage of those positive at one or the other examination from 55·2 to 68·1. In a group of fifty-one adults at Yaba, four monthly examinations (October to January) raised this percentage from 43·1 to 92·2. It is probable that there are very few individuals in the average Nigerian village or town who do not have parasites in detectible numbers in the peripheral blood at some time during the year.

Type of malaria parasite.—We examined a large series of thin films stained by the Shute modification of Leishman's stain as described by James (1929). In young parasites this stain clearly differentiates Maurer's dots, characteristic of malignant tertian parasites. It is largely on this evidence that we have concluded that the great majority of quartan-containing specimens have

malignant tertian parasites as well, and that the malignant tertian parasite is nearly universal in Southern Nigeria. We found about a dozen infections with benign tertian parasites, all or the most of which seemed to be *Plasmodium ovale* Stephens. The red blood cell was but little enlarged, pigment was sparse, chromatin tended to divide early, and sporulation in one specimen at least resembled that of quartan. Schüffner's dots were well marked in all.

Aside from this possible variety we detected no type of malaria parasite which we have not also found in the Western Hemisphere. The proportion of malignant tertian and quartan parasites in the population of Southern Nigeria was about the same as that which we have found among negroes in Haiti.

Intensity of parasitic infestation.—A statistical analysis of the changes in the mean intensity of malarial infestation with age in our series of blood examinations has been prepared by Dr. Persis Putnam of the Rockefeller Foundation, and is included in the Appendix to this paper. This analysis is based on the numbers of parasites per thick-film field. It indicates that the mean infestation rate rises steadily from the age 3 months to the age 2 years, then drops rapidly to age 16 years and over. It follows, then, in a general way, the results shown in the seventh column of Table I, which gives merely the percentage of individuals in each age group which showed one or more parasites per thick-film field.

The rapid diminution in the infestation index from age 2 years upwards shows that individuals acquire some property which inhibits the free multiplication of parasites in the peripheral blood. We cannot, however, assume that infants under 2 years of age possess this or any inhibiting property which subsequently diminishes or is lost; for these infants are receiving many new infections not only with quartan or any fresh species of Plasmodium, but possibly with many biological types of different species as well. Until we can measure the effect of these new infections, we cannot assume the presence of any resistance coming from the mother before birth or through lactation, an attractive theory because mothers have certainly acquired something which limits the growth of parasites in their own blood, and one might expect it to be transmitted.

Some inhibition to the multiplication of parasites in the peripheral

blood, often associated with a tolerance to small numbers of parasites, follows early attacks at all ages in different races of man and in the malaria of lower animals as well. We probably have nothing in West Africa that is not found in any hyperendemic area where first attacks come very early in life, and where mothers have been infected since their infancy. It is noteworthy that in our group we find a rough parallel of general parasitic infestation and crescent infestation. The crescent rate falls as 'immunity' is established. In this our results agree with those of Christophers and Sinton in India.

Analysis by locality and season of the parasite indices of infants and mothers. The results obtained with infants (Tables I and II) are worthy of further analysis. These infants are virtually newcomers to the region, little protected by mechanical or other measures against infection. They offer a good index of the malarial danger of a locality or season.

In Table III (fig.)1* we show by month and locality the parasite index of infants 3 and 4 months old brought to the Health Offices of Lagos and Ebute Metta for vaccination. They form an unselected group—clinical cases are excluded from this Table—and their ages are definitely known. We have included in this age group a few infants whose age was given as $4\frac{1}{2}$ months, and in the Ebute Metta group a very few picked up in near-by villages, but not selected on account of illness.

The parasite index of mothers who brought these infants to the health offices is compared. The period of observation of Lagos infants extended from November, 1929, to December, 1930, inclusive; that of Ebute Metta infants and of all mothers is shorter.

It appears that the parasite index of Lagos infants reached its maximum in September, apparently during the first half of the month (see footnote to Table III). The high rate observed during December, 1929, is hard to explain, since the lowest point in the curve is reached in December, 1930. The numbers examined during December, 1929, are small, but the rate during January, 1930, was also high, indicating that there was some factor present during that period which was less effective a year later, possibly the number of *Anopheles*. Probably

* Figures 1, 2 and 3 have been plotted on a semi-logarithmic scale. This was done to bring out the percentage changes in the various indices from month to month rather than the actual amount. The slope of the line indicates the percentage of rise or fall of the index from one point of observation to another. When different indices run parallel courses, the monthly percentage changes are similar.

TABLE III.

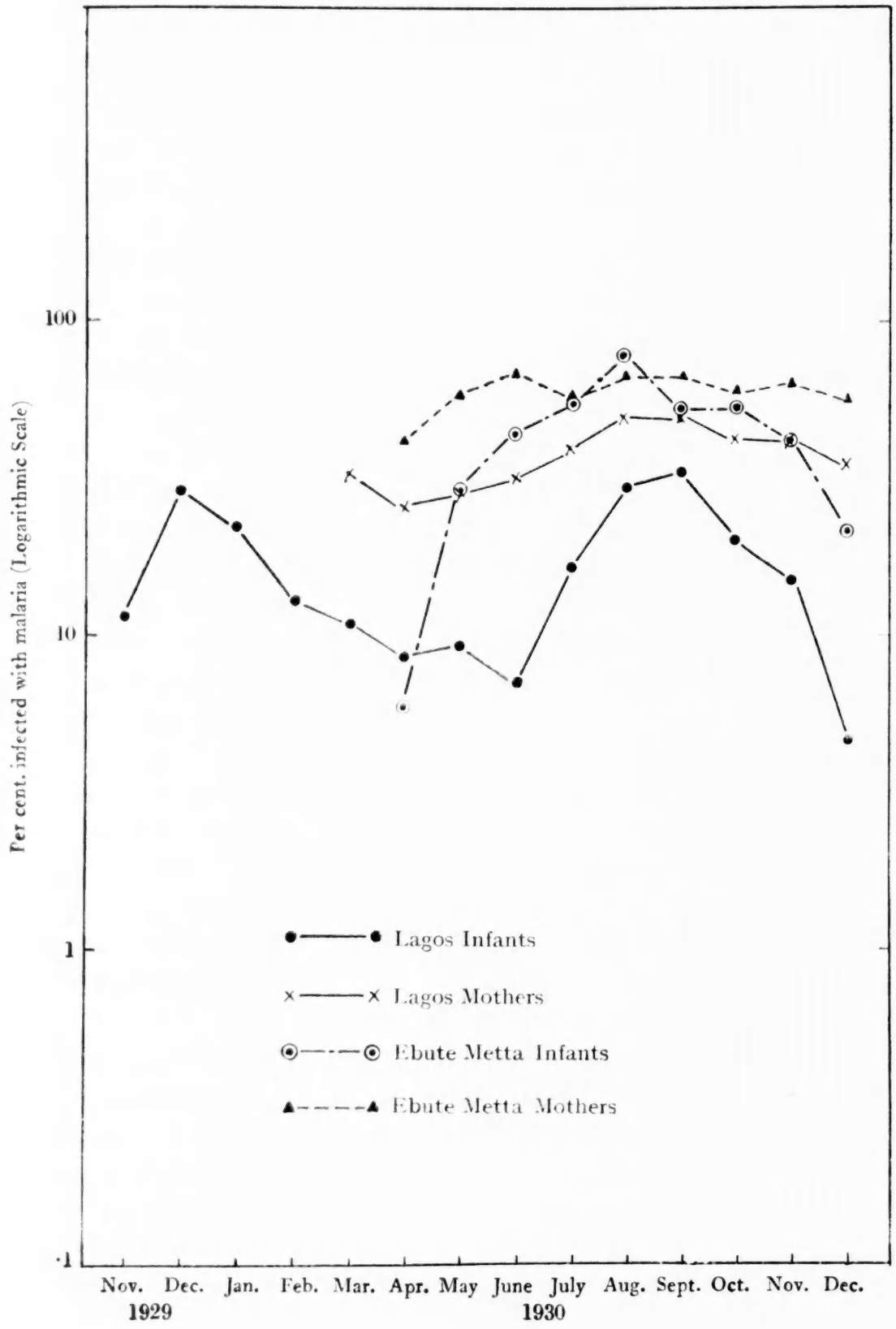
Malaria parasite rate of infants 3 to 4 months old and of mothers of infants of that age. By month and locality.

Year and month	Health Office, Lagos						Ebute Metta and Vicinity					
	Infants			Mothers			Infants			Mothers		
	Number examined	Number positive	Percentage positive	Number examined	Number positive	Percentage positive	Number examined	Number positive	Percentage positive	Number examined	Number positive	Percentage positive
1929												
November	17	2	11·8	...	...	...	...	...	...	...	...	...
December	38	11	28·9	...	...	...	...	...	...	...	...	...
1930												
January	77	17	22·1	...	...	...	...	...	...	...	...	...
February	62	8	12·9	...	...	...	...	...	...	...	...	...
March ...	83	9	10·8	54	17	31·5	...	...	...	...	...	...
April ...	83	7	8·4	88	22	25·0	17	1	5·9	17	7	41·2
May ...	66	6	9·1	72	20	27·8	38	11	28·9	33	19	57·6
June ...	74	5	6·8	78	24	30·8	28	12	42·9	23	15	65·2
July ...	98	16	16·3	93	36	38·7	34	18	52·9	29	16	55·1
August ...	98	28	28·6	83	39	47·0	47	35	74·5	45	29	64·4
*September	113	36	31·8	109	52	47·7	40	20	50·0	39	25	64·1
October...	140	27	19·3	138	57	41·2	36	18	50·0	35	20	57·1
November	94	14	14·9	85	34	40·0	22	9	40·9	21	13	61·9
December	65	3	4·6	65	22	33·8	19	4	21·1	17	9	52·9
All months	1,108	189	17·1	865	323	37·3	281	128	45·6	259	153	59·1
* September, 1930, is divided as follows :												
1st half month	52	23	44·2	50	27	54·0	23	11	47·8	23	15	65·2
2nd half month	61	13	21·3	59	25	42·4	17	9	52·9	16	10	62·5

FIGURE 1

Comparison of malaria parasite rates of infants 3 to 4 months old and of their mothers by month and locality

(Table III)



the only peak in the curve which occurs every year is that of the third quarter of the year. The index during October and November may be increased by infants who became infected during their pre-vaccination period in July and September when malaria was abundant. This hypothesis is supported by the results obtained at the re-examination of about 100 infants of Lagos and Ebute Metta in all cases at least a month after their first examination. It appeared that among sixty positive at their first examination 85 per cent. were still positive at their second ; while of forty-one negative at first examination only 43 per cent. remained negative.

Positives, then, tend to remain positive, so that the parasite rate would tend to cumulate after a period of high malaria prevalence. Further, since so large a proportion (57 per cent.) of the negatives later became positive, it is probable that nearly all infants are infected before they are a year old.

In a general way, the curve of the infant parasite index tended to run parallel with that of the anopheline infection rate of the whole region in and about the two cities (Table V). The abundance of *Anopheles* was, of course, also an important factor. The rise in the infant parasite rate began about a month earlier than that of the anopheline infection rate and at a time when the anopheline density was high.

The parasite index of Ebute Metta infants is based on relatively small numbers and tends to be irregular. However, it follows roughly the same seasonal curve as that of Lagos infants but on a much higher level.

The adult parasite index, as represented by mothers of infants, is much higher than that of infants but tends in the Lagos group to run parallel with the infant curve. Ebute Metta mothers show little indication of seasonal variation, possibly because they appoach the malaria saturation point at all times.

We have here two groups of adults of like social status and both resident in large towns. In Lagos the anopheline infection rate is high but anopheline density low ; in Ebute Metta both infection rate and density are very high. The infant malaria parasite rates give a fair index of the degree of exposure to malaria infection in the two localities. The blood parasite rate of Ebute mothers is much higher in every month than that of Lagos mothers. The totals

of all months are: Ebute Metta mothers, 259 examined, 59·1 per cent. positive; of Lagos mothers, 865 examined, 37·5 per cent. positive. It would appear from this evidence that the parasite index of African adults is affected by frequent reinfection in spite of the fact that they have been subject to occasional infection since their infancy, and probably have been carriers of parasites all their lives. Ebute Metta is, as regards exposure to malarial infection, more typical of communities in Southern Nigeria than is Lagos.

II. THE EFFECT OF MALARIAL INFECTION ON THE HEALTH OF AFRICANS

We have seen in the preceding section that the malaria parasite index and the degree of infestation are very high at all ages up to the adult. There is little evidence of a racial immunity against infection except possibly in the low infection index shown by infants 4 months old or younger. This index, only 22·2 per cent. in all infants, hardly exceeded 45 per cent. in Ebute Metta, a locality where infected *Anopheles* were especially abundant. One would expect in this locality a fairly large percentage of human infections after a residence of 4 days, and 4 weeks would seem to be ample for the infection of every infant. Infants may be better protected by clothing or night covering against the bites of mosquitos than are older children, but this explanation seems hardly adequate.

A racial tolerance to the effects of malaria parasites is another matter. We give here the few data we have obtained regarding the effect of malaria on the health of individuals.

The spleen index of about 1,600 school children of Lagos was determined by Dr. W. C. Smith, School Medical Officer of Health of Lagos, and the malaria parasite index of the same children by ourselves. The spleen index was lower than the parasite index in this group. It was definitely higher among malaria-positive children than among those malaria-negative. A more detailed report on these examinations will be given by Dr. Smith.

Anaemia as detected in thick-film blood examinations is relatively high among infants and young children. We have not adequate data for comparison, but have a distinct impression that the anaemia rate among African children is much lower than that which we have found among white children equally parasitized.

Temperature. The rectal temperatures of 205 infants, 3 or 4 months of age, coming to the Health Office of Ebute Metta for vaccination, were taken by one of us (M. T. Olinger). The results are shown in Table IV. It appears that there was no marked correlation of temperature and parasites below 38° C. Above that point the proportion of infants who were malaria positive tends to increase until at 38·2° and above, all infants were parasitized. Very high temperatures, then, were always associated with malaria infection. The malaria index is based on the examination of a single thick film, and undoubtedly many negatives were really carrying parasites in small numbers. Further, many of the positives with normal temperature may have had fever at their first attack, although some of them were still carrying large numbers of parasites. On the whole, we found among these infants little evidence of a continuous high temperature associated with the presence of malaria parasites. For a comparison of parasite index and temperature in African school children, see the work of Macdonald (1926).

TABLE IV

Infants 3 and 4 months old appearing at the Health Office of Ebute Metta for vaccination. Rectal temperatures and percentage with malaria percentages in the blood

Rectal temperatures ...	36·6 to 36·7	36·8 to 36·9	37·0 to 37·1	37·2 to 37·3	37·4 to 37·5	37·6 to 37·7	37·8 to 37·9	38·0 to 38·1	38·2 to 41·1	TOTAL
Numbers examined ...	2	5	38	52	56	21	11	6	14	205
Per cent. with parasites	0·0	80·0	52·6	48·1	42·8	52·4	45·5	83·3	100	43·9

Clinical symptoms among infants. At the time of examination clinical symptoms among infants seemed to be generally lacking, whether the infant was malaria positive or not. Of 163 noted as 'O.K.' by the examiner (M. T. Olinger), seventy-three were malaria positive, some of them with enormous numbers of parasites. A large proportion of those who exhibited restlessness, coryza, hot skin, or tender spleen proved to be malaria positive. On the other hand, only about one out of five emaciated infants was positive; if they had been infected previously the parasites had disappeared

Six infants who were ill at the time of their first examination were kept under observation by one of us (M. T. Olinger). All then had large numbers of parasites in the blood except one, who later became positive. At the end of the period of observation, which extended over a period of 6 to 10 months, all of the infants were apparently well, although at the time of the last examination, late January, 1931, three, whose blood specimens were obtained, still had many parasites and marked anaemia. The mothers of these six infants were given quinine to administer to them; whether it was given regularly, or whether these small doses did any good or not is difficult to determine.

Deaths among infants. We have data on only 268 infants, all of whom we examined at the Ebute Metta Health Office. Our examination of these infants began April 17th, 1930. The records up to January 28th, 1931, show that nine, or 3·3 per cent., had died. We have no record of nineteen who had moved from Ebute Metta to some other locality. The percentage of these infants malaria positive at first examination was approximately 45·0, and it is probable that nearly all of them became infected before the year was over.

Clinical symptoms among school children. These are probably not uncommon, according to information given us by teachers familiar with the symptoms of malaria.

Clinical symptoms among adults. We have records of twenty-two cases, probably malaria, occurring during a period of a little over a year among the employees of the West African Yellow Fever Commission at Yaba, a personnel averaging about eighty. In all of these cases malaria parasites were found in the blood, and, what is more significant, in fifteen of them the parasites were more numerous than one per thick-film field. In our surveys of the malaria parasite index of this group we have found infestation of that degree in only about 3 per cent. In nearly half of these cases a high temperature, reaching in some to 103°, is recorded. Symptoms such as chills, headache, and various pains were common, and, in a few cases, sweating, and tenderness of the spleen. There seems to be no doubt that a part, and probably the majority, of these cases were malaria.

In sum, it would appear that Africans of this region never acquire a complete tolerance to the effects of malaria parasites, at least not up to the period of middle age. Whatever tolerance is enjoyed by

adults must be slowly acquired. Nearly all of them are infected in infancy and receive during adolescence countless bites of mosquitos infected with whatever species or race of *Plasmodium* may be present in the region. We assume that children suffer far more with the disease than do adults, but it is not easy to measure the difference. A large percentage of infants and nearly all children one year of age and older carry parasites, usually in large numbers. Whether an illness or a death is due to malaria or not is difficult to determine. With regard to the question of racial tolerance to the effects of infestation among negroes of Southern Nigeria, it would seem that comparison with other races equally exposed to infection and equally well nourished is necessary to a decision.

III. THE MALARIA PARASITE RATE OF *ANOPHELES* COLLECTED IN AFRICAN DWELLINGS

Our chief purpose in this survey was to compare the natural anopheline infection rate of different localities and seasons. The description of the technique employed and of some interesting side-products of the investigation will be dealt with in a separate paper. In order to secure a more or less uniform distribution of error, all specimens were examined and nearly all dissections done by one person (M. A. Barber). We made the series as large as possible in order to reduce the error of small numbers where localities or months are compared.

A. Coast Region in the Vicinity of Lagos. *A. costalis.*

Anopheles for dissection were collected by our own assistants and those of Dr. Joseph Cauchi, Medical Officer of Health, Lagos, who generously assisted us in this work. The anopheline density index (Tables V and VI) is measured by the average number of *Anopheles* per room. All species and both sexes of *Anopheles* were included in estimating this index. The great majority were females and the proportion of species other than *A. costalis*, insignificant. This index is based on numbers much greater than the numbers dissected. In determining the index shown in Table V, over 25,000 were counted, and in that of Table VI, over 13,000. Only collections made by one of us or by one African collector were used in estimating the density

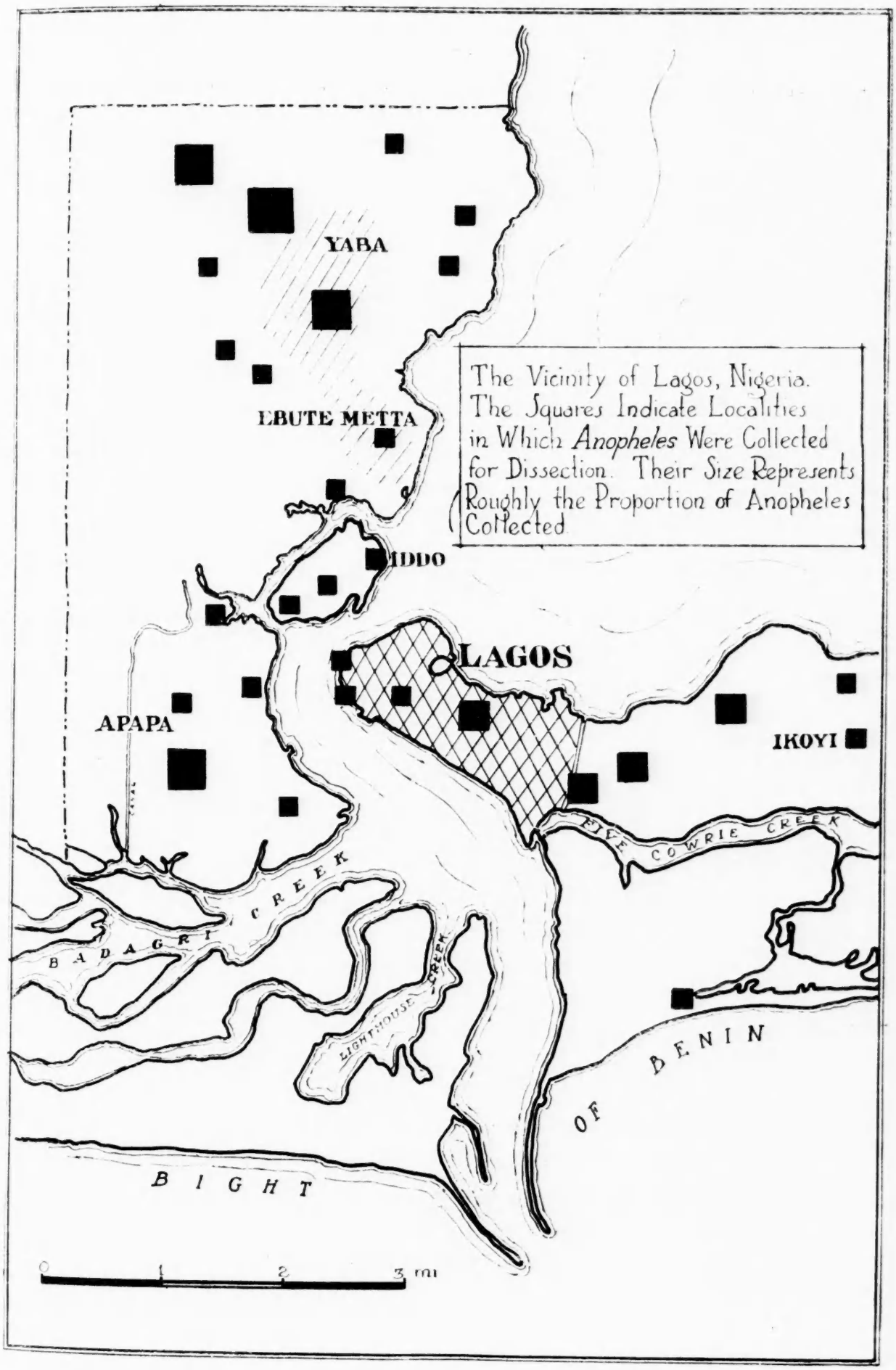
index. The African collector was unusually skilful in finding *Anopheles* and reasonably reliable in his reports; but one of us accompanied him on many trips and occasionally checked his results by re-examining rooms. In any case, only the larger variations in density are taken into consideration in comparing seasons or localities.

Among dissections, only those obtained with one species of *Anopheles*, *A. costalis*, are included. The results are shown by months in Tables V and VI and in fig. 2. These include the anopheline density index, and Table V the monthly rainfall of Lagos as well. Table V and fig. 2 cover all localities in which *Anopheles* were collected for dissection. With the exception of one locality, Otta Town, about 20 miles distant, all collections were made within 8 miles of the centre of Lagos. (See map, page 475.) Table VI covers but one locality, the compound of the Yellow Fever Commission at Yaba. Here the error of unequal selection of localities is excluded.

The dissections were continued through 19 successive months, July, 1929, to January, 1931, inclusive. Comment on the results will follow in sub-section B, which treats of similar work in Ibadan, Nigeria.

In Table VII the results appearing in Table V are arranged by localities and include only dissections made up to January 1, 1931. Localities are listed in the order of the total infection rate except 'miscellaneous,' which includes a large number of localities in each of which less than one hundred dissections were done. The anopheline intensity index is shown only for localities in which large collections were made by ourselves or by our own collector.

The variation in the anopheline infection rates of different localities is striking. The differences were so great that we had to apportion to various localities a proper number of monthly visits in order to get a fair comparison of seasonal variation. In general, large towns and other thickly populated places (e.g. Otta Town, Yaba, Idioro) gave the higher rates; while more isolated localities (e.g. Apapa, Ikoyi Swamp Villages, Onikan Road) gave the lower rates, although some of these are situated in the immediate vicinity of large breeding grounds of *A. costalis*. There is but little evidence of correlation of anopheline infection and anopheline density. Population density seems to be the more important factor.



The Vicinity of Lagos, Nigeria. The Squares Indicate Localities in Which *Anopheles* Were Collected for Dissection. Their Size Represents Roughly the Proportion of Anopheles Collected

TABLE V

Malaria parasite index of *A. costalis* collected in native houses in the Coast Region of Southern Nigeria, all localities, by months

Month	Total infection rate, stomach or salivary glands			Sporozoite rate, salivary glands			Anopheline density			Rainfall Lagos, inches
	Number examined	Number positive	Percentage positive	Number examined	Number positive	Percentage positive	Number collected	Number rooms	Rate per room	
1929										
July	20	2	10·0	19	1	5·2	20	11	1·8	19·9
August ...	348	57	16·3	342	34	9·9	505	217	2·3	0·8
September ...	331	75	22·6	315	44	13·9	279	180	1·5	3·1
October ...	406	33	8·1	402	20	4·9	362	207	1·7	6·0
November ...	622	36	5·7	614	15	2·4	350	271	1·2	4·1
December ...	1,102	66	5·9	1,095	25	2·2	1,148	364	3·1	6·0
1930										
January ...	885	94	10·6	860	37	4·1	1,659	205	8·0	1·4
February ...	673	85	12·6	665	51	7·6	1,345	212	6·3	2·2
March ...	799	55	6·8	792	24	3·0	1,375	311	4·4	3·3
April ...	945	95	10·0	932	47	5·0	1,802	427	4·2	5·0
May... ...	1,328	121	9·1	1,315	61	4·6	3,463	334	10·3	8·6
June... ...	1,191	118	9·9	1,188	68	5·7	2,998	189	15·8	13·3
July	1,475	159	10·7	1,453	55	3·7	3,503	251	13·9	18·4
August ...	1,355	321	23·6	1,339	211	15·7	1,572	327	4·8	0·7
September ...	731	187	25·5	725	117	16·1	927	274	3·3	2·7
October ...	959	85	8·8	956	40	4·1	979	331	2·9	13·1
November ...	736	91	12·3	730	54	7·3	948	315	3·0	1·9
December ...	794	88	11·0	769	48	6·2	1,677	342	4·9	1·7
1931										
January ...	444	52	11·7	442	31	7·0	444	182*	2·4	—
All months ...	15,144	1,820	12·0	14,953	983	6·6	25,356	4,950	5·1	6·2 in. monthly

* First half of the month

TABLE VI

Malaria parasite rate of *A. costalis* collected in native houses in Southern Nigeria
Compound of Yellow Fever Commission, Yaba, by months

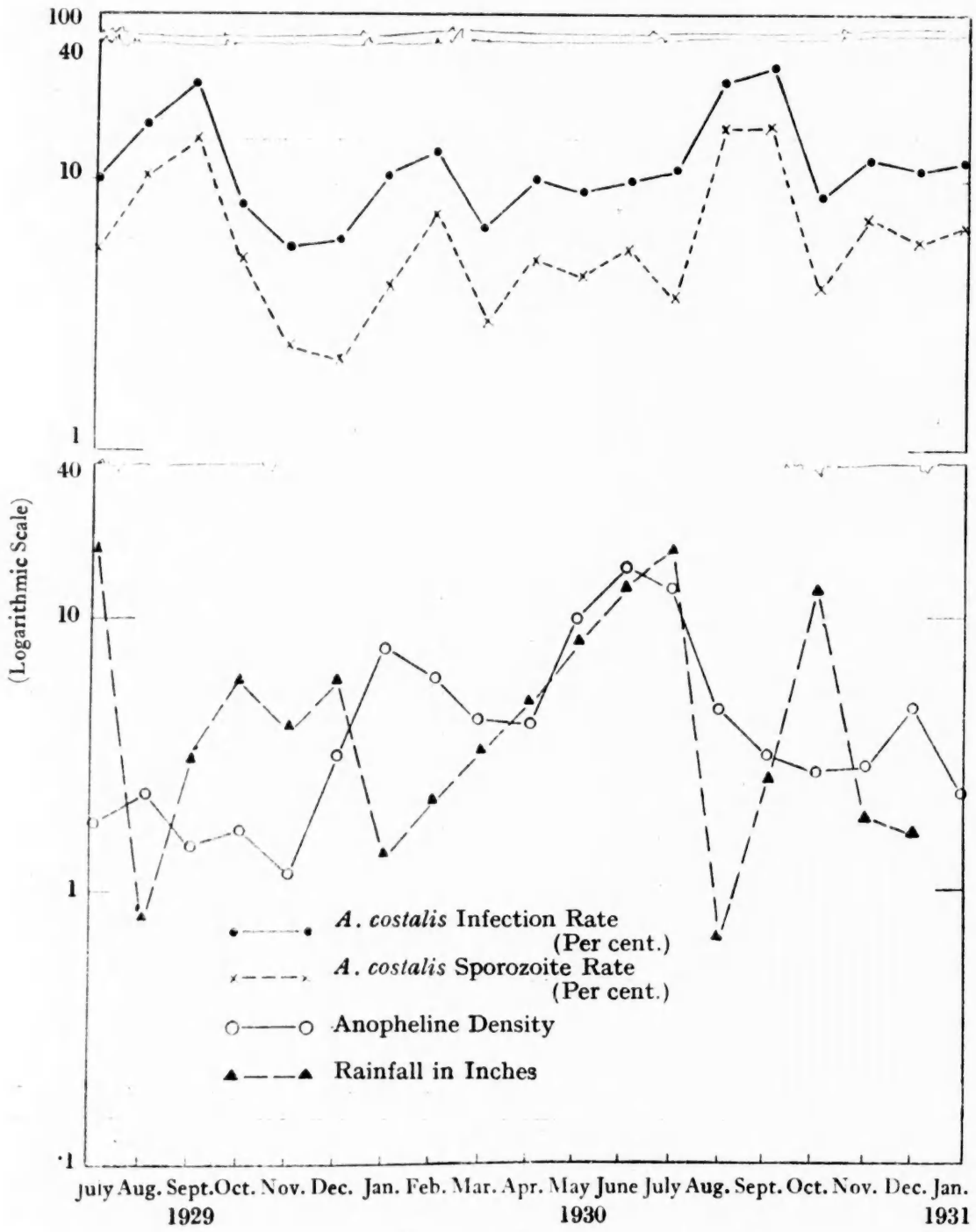
Month	Total infection rate, stomach or salivary glands			Sporozoite rate, salivary glands			Anopheline density		
	Number examined	Number positive	Percentage positive	Number examined	Number positive	Percentage positive	Number collected	Number rooms	Rate per room
1929									
July	19	2	10·5	18	1	5·6	20	11	1·8*
August ...	123	26	21·1	123	17	13·8	336	119	2·8
September ...	138	24	17·3	127	14	11·0	194	147	1·3
October ...	152	18	11·8	148	10	6·7	275	189	1·4
November ...	179	23	12·8	178	9	5·0	253	266	0·9
December ...	477	32	6·7	474	8	1·6	721	275	2·6
1930									
January ...	630	67	10·6	612	25	4·0	1,161	160	7·2
February ...	434	73	16·5	428	44	10·2	806	142	5·6
March ...	296	27	9·1	292	12	4·1	367	150	2·4
April ...	456	41	8·9	447	18	4·0	639	144	4·4
May... ...	584	57	9·7	576	28	4·8	1,565	153	10·2
June... ...	512	50	9·7	510	23	4·5	1,653	86	19·2
July	653	62	9·4	644	16	2·4	1,886	117	16·1
August ...	534	96	17·9	522	60	11·4	853	85	10·0
September ...	276	69	25·0	273	39	14·2	420	124	3·3
October ...	277	20	7·2	277	11	3·9	413	145	2·8
November ...	220	20	9·1	218	11	5·0	361	141	2·5
December ...	400	47	11·7	399	25	6·2	1,150	204	5·6
1931									
January ...	181	16	8·8	180	12	6·7	126	65	2·0*
All months ...	6,541	770	11·8	6,446	383	5·9	13,201	2,723	4·8

* First half of the month

FIGURE 2

Malaria parasite rate of *A. costalis* collected in native houses compared with Anopheline density and rainfall, by months

Vicinity of Lagos, Coast Region (Tables V and VI)



B. Ibadan

Ibadan is situated about 100 miles north of Lagos and is wholly inland. It is a hilly region about 600 feet above the sea, and is subject to a winter climate somewhat different from that of the coast. According to the Nigerian Handbook, 1929 edition, the rainfall in November, December, and January is 1·91, 0·79 and 0·57 inches, respectively (average of 24 years' observation), while that of Lagos for the corresponding months is 2·73, 0·89 and 1·21 (average of 28 years of observation). The incidence of the species of *Anopheles* differs somewhat from that of the coast—*A. funestus*, for example, being more abundant in the hills. So it seemed worth while to compare some of our coast observations with a series in Ibadan. Further, our collections for dissection of *Anopheles* on the coast were made in various localities, many of them in small villages, while those of Ibadan were all made in the midst of a large city of about 250,000 inhabitants.

Our observations extended over a period of 6 months, August, 1930, to January, 1931. The *Anopheles* were collected under the direction of Dr. A. W. Burke, of the West African Yellow Fever Commission, from African houses in the Oke Padi quarter of the city, and were sent to Yaba for dissection. The breeding of *Anopheles* is common in several streams flowing through Ibadan.

The results of the *Anopheles* dissections appear in Table VIII and in fig. 3 are compared with those obtained at the coast.

It will be noted in Table VIII that the infection rate of *A. costalis* is higher than that of *A. funestus* in the totals and in nearly every month. That of *A. hargreavesi* is lowest of all, but we have but few numbers on which to base the percentage. In the vicinity of Lagos approximately 3 per cent. of this species have been found positive among nearly 228 dissected, the majority of the positives showing sporozoites in the salivary glands. Among seventy specimens of *A. funestus* collected in the vicinity of Lagos we found 15·7 per cent. positive, about the same rate as that of Ibadan.

In fig. 3 only *A. costalis* is included. It appears that both the general infection rate of this species and the sporozoite rate in Ibadan tend to follow somewhat the same monthly variation as they do in the vicinity of Lagos, but at a higher level. The numbers on which the coast percentages are based follow: August, 1,355 dissected; September, 731; October, 959; November, 736; December, 794; January, 442.

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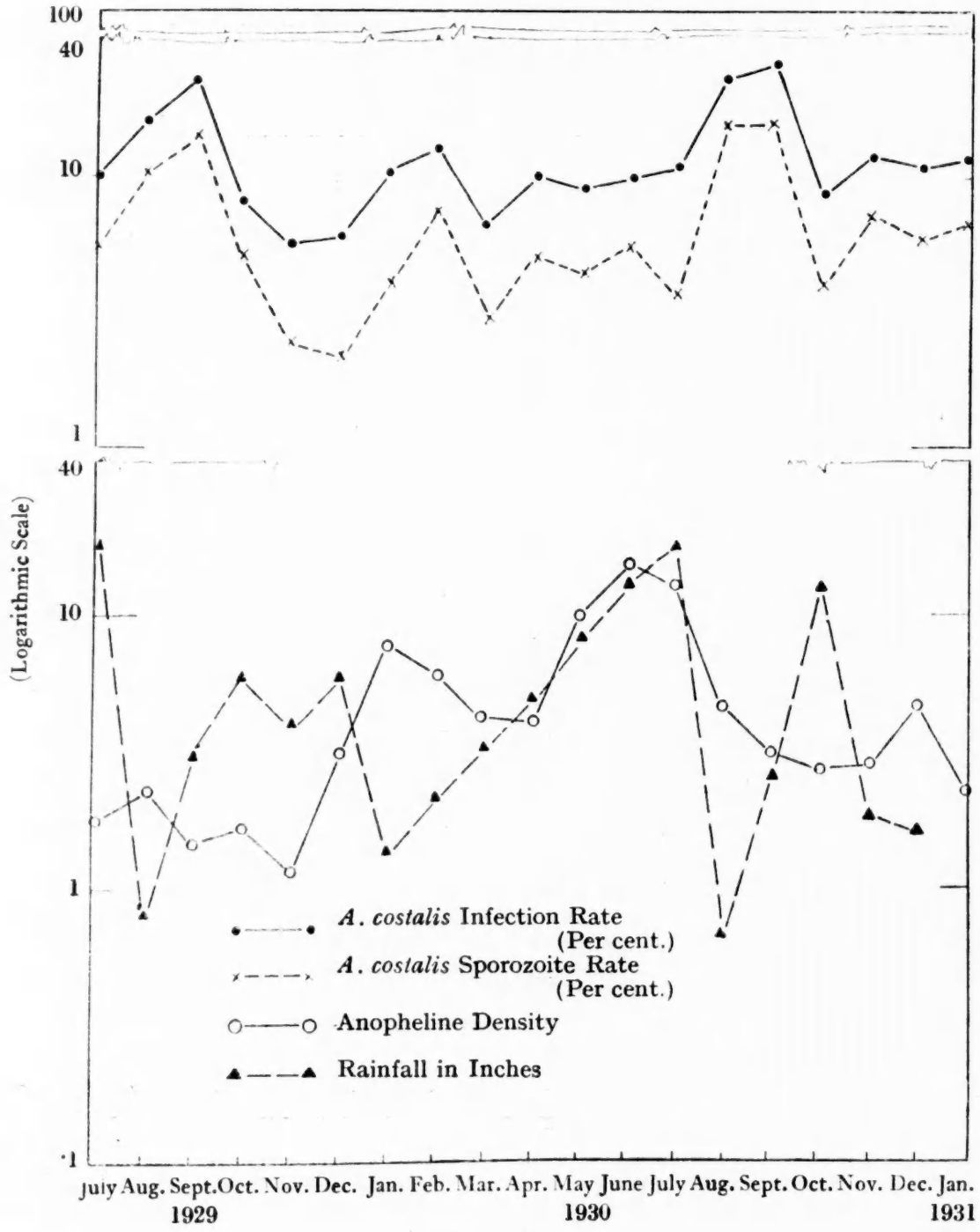
Malaria parasite rate of *A. costalis* collected in native houses compared with Anopheline density and rainfall, by months

Vicinity of Lagos, Coast Region (Tables V and VI)



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The rainfall of Lagos and that of Ibadan from July, 1930, to December, 1930, inclusive, are also shown in fig. 3, and the complete meteorological data of Ibadan for 1930 are given in the Appendix to this paper. Anopheline density data, based on the rate per room, are also shown in fig. 3, those of Ibadan being

TABLE VII

Malaria parasite index of *A. costalis* collected in native houses in the Coast Region of Southern Nigeria, by localities

Locality	All infections, stomach or salivary glands			Sporozoite rate, salivary glands			Anopheline density
	Number examined	Number positive	Percentage positive	Number examined	Number positive	Percentage positive	Rate per room
Otta Town	202	82	40·0	200	61	30·5	2·0
Yaba Police Barracks...	1,580	283	17·9	1,570	181	11·5	8·6
Idioro	1,377	228	16·5	1,364	119	8·7	6·1
Ikoyi Soldiers' Barracks	803	106	13·2	778	70	8·9	—
Yaba, Boys' Houses ...	6,453	765	11·8	6,359	380	5·9	4·6
Okesuna Police Barracks	395	40	10·1	393	20	5·1	—
Apapa Police Barracks	290	26	8·9	288	8	2·7	7·1
Iddo Island	118	10	8·4	120	4	3·3	1·1
Ikoyi Boys' Houses ...	357	29	8·1	355	10	2·8	—
Ikoyi Police Barracks...	753	60	7·9	749	28	3·7	—
'Jack-ashore'... ...	199	11	5·5	193	5	2·6	7·4
Apapa Boys' Houses ...	1,278	70	5·5	1,269	32	2·5	8·7
Onikan Road	341	16	4·7	336	8	2·3	—
Ikoyi Swamp Villages	327	11	3·3	318	7	2·2	7·6
Miscellaneous ...	431	61	14·1	418	36	8·6	0·5
TOTALS ...	14,904	1,798	12·6	14,710	969	6·6	4·8

taken from Table VIII and those of the coast from the average of a number of collecting places.

We examined blood specimens (thick film) of 104 persons of Ibadan, all, or nearly all, school children over 4 years of age. We found 101, or 97·1 per cent., positive for malaria parasites.

TABLE VIII

Result of dissection of *Anopheles* collected at Ibadan, Nigeria, August, 1930, to January, 1931
Oke Padi Quarter

	Total infections, stomach or salivary glands			Salivary glands, sporozoite rate		
	Number dissected	Number positive	Percentage positive	Number dissected	Number positive	Percentage positive
			A. costalis			
1930						
August ...	215	56	26·0	214	33	15·4
September ...	100	30	30·0	100	18	18·0
October ...	46	11	23·9	46	6	13·0
November ...	67	14	20·9	65	8	12·3
December ...	147	21	14·3	146	13	8·9
1931						
January ...	178	31	17·4	177	20	11·3
TOTAL, all months ...	753	163	21·6	748	98	13·1
			A. funestus			
1930						
August ...	104	17	16·3	102	8	7·8
September ...	52	9	17·3	50	4	8·0
October ...	80	9	11·3	80	4	5·0
November ...	42	5	11·9	41	2	4·9
December ...	20	3	15·0	20	2	10·0
1931						
January ...	11	1	9·1	11	1	9·1
TOTAL, all months ...	309	44	14·2	304	21	6·9
			A. hargreavesi			
1930						
October ...	3	0	0·0	3	0	0·0
November ...	5	1	20·0	5	0	0·0
December ...	7	0	0·0	7	0	0·0
1931						
January ...	27	1	3·7	27	0	0·0
TOTAL, all months ...	42	2	4·8	42	0	0·0

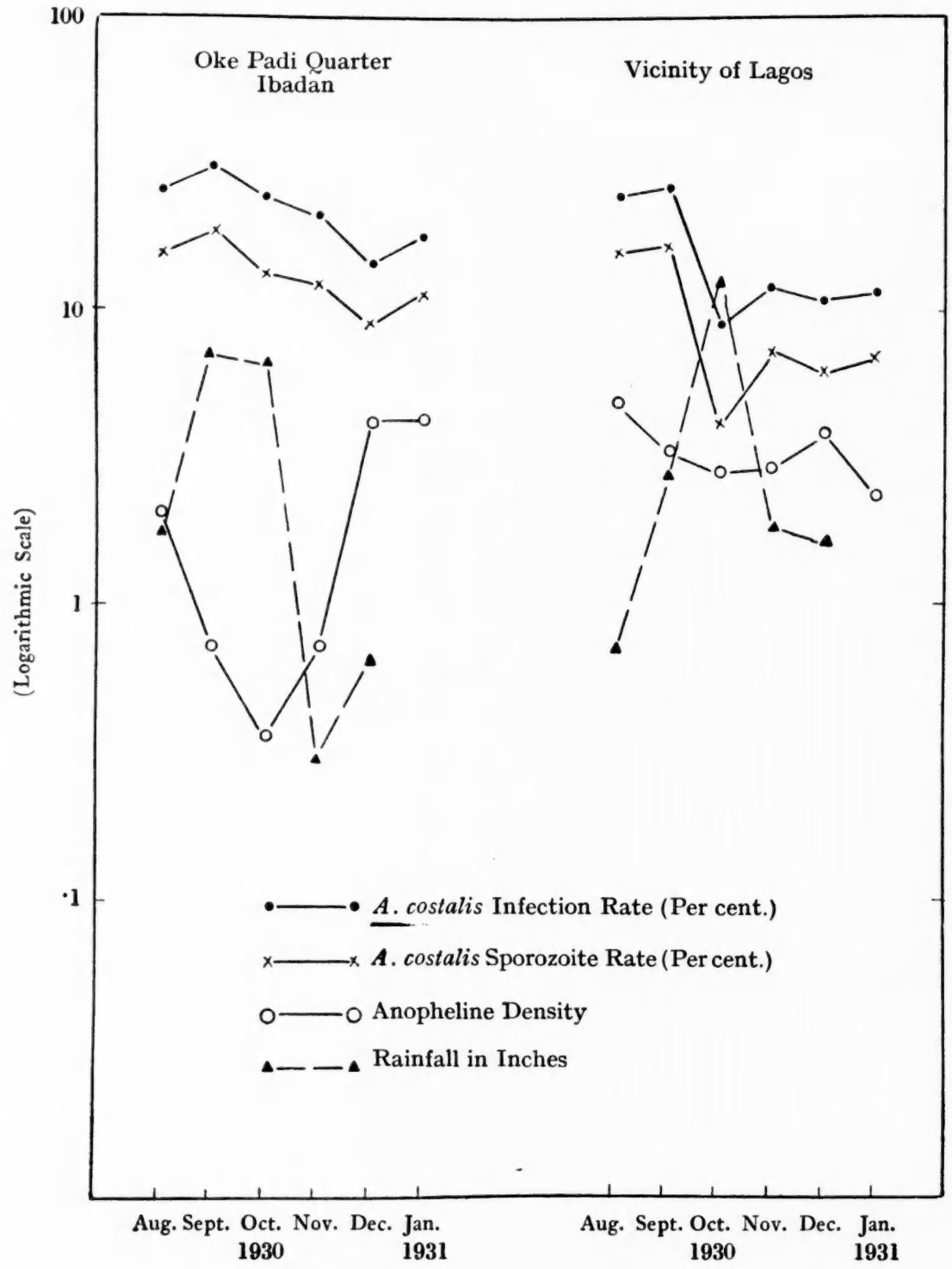
All species, Anopheline density

Month	Number of *Anopheles* collected	Number of rooms inspected	Rate per room
1930 August	490	239	2·10
September ...	195	269	0·72
October	167	469	0·36
November ...	196	268	0·73
December ...	217	52	4·17
1931 January	339	81	4·18
TOTAL, all months ...	1,604	1,378	1·16

FIGURE 3

Malaria parasite rate of *A. costalis* collected in native houses compared with Anopheline density and rainfall, by months

Oke Padi Quarter, Ibadan and Coast Region, Vicinity of Lagos (Table VIII)



C. Discussion of the Seasonal Variations in the Anopheline Infection Rates as Observed in the Coast Region and in Ibadan

Meteorological conditions should be an important factor in determining the seasonal variation of anopheline infection. They may function, theoretically, in three ways: (1) by increasing anopheline density; (2) by affecting the longevity of *Anopheles* and the rate of growth of malarial parasites in them; (3) by increasing the proportion of human gametocyte carriers—' unhealthy' weather favouring malarial relapse.

Meteorological tables for Lagos and Ibadan are given in the Appendix to this paper. These include data of both localities for the period 1920-1929, inclusive, and the year 1930. In addition, those of Lagos, 1929, are included because part of our survey was done during that year. These data were kindly furnished us by Mr. J. Calder Wood, Surveyor-general of Nigeria, and his assistants.

It appears from these data that there is a dry season in both localities especially marked in December, January, and February, and a wet season culminating in June. There is a remission of rainfall during August, which in 1930 was more marked than in average years. In that year we could properly speak of a dry season in August followed by a period of ' little rain.' The rainfall in Lagos during the period covered by our survey may be briefly outlined as follows: In 1929, heavy rainfall in May, June, and July, very light in August, and moderate (3 to 6 inches) during the rest of the year. In 1930, gradually increasing rainfall from January to July, very slight in August, increasing in September, very high in October, and very little in November and December.

The anopheline density of the vicinity of Lagos as shown by Tables V and VI was very high in the rainy period, May to July, 1930, then fell rapidly in August. The variations during the rest of the year are of less significance.

The curve of the anopheline infection rate, all localities, has a high peak in September of both 1929 and 1930. It rises rapidly during August, a month during which the anopheline density was falling. This is what we would expect, since with a diminishing anopheline population the average age of individuals should rise and

the period of exposure to gametocyte carriers should increase. By the same reasoning we should expect a fall in the mosquito infection rate during the period, April to June, 1930, because then rains were increasing and the anopheline density rising. The mosquito population should be then diluted by many non-infected newcomers. The anopheline infection rate, however, remained at nearly a constant level from April to July. Evidently the infection curve in our survey did not always run true to form.

It is to be remembered, however, that rain fell during every month of our survey. Further, there are ponds and large coastal swamps in the region surrounding Lagos in which anopheline production never ceases. Changes in anopheline density are gradual, and the range of dispersion wide. We would not expect the close relation between anopheline infection and rainfall such as is found in drier regions with sharply marked wet and dry periods. Compare the work of Taylor at Gadau, Northern Nigeria (1930). Again in Southern Nigeria we deal with a human population very dense and nearly saturated with malaria, a factor which might tend to keep the anopheline infection rate at a more constant level.

In the interpretation of our results we must make much allowance for variations not constant from year to year. We note that the infection rate of *Anopheles* was very low during November and December, 1929, while during the same months of 1930 it was above the average. Probably the only peak which occurs every year is that of September.

In Ibadan the anopheline infection curve follows the same general trend as that of the coast region—a peak in September followed by a decline. In both regions there were heavy rains in June and July with little rain in August.

The temperature and relative humidity of the coast region of Nigeria must rarely be unfavourable to adult *Anopheles*. There is nothing in our results to indicate such effect, except possibly the behaviour of the infection curves (general infection and sporozoite) in December, 1929. These were low in that month, the sporozoite index reaching its lowest level in the whole series ; while they were above the average level in December, 1930. In December, 1929, the dry harmattan wind prevailed, while in 1930 it was lacking and the weather was hot and relatively humid. We may have here

merely a coincidence of two conditions, certainly no conclusive evidence of cause and effect.

The theory that weather unfavourable to the health of the human population may affect the gametocyte rate through relapse of malaria seemed worthy of consideration in Southern Nigeria. Cooler weather prevails there in August and September and is associated with increased illness among the African people; while October, November, and December are relatively healthful months. The anopheline infection rate rises to a maximum in September and falls rapidly in October. During the third and fourth quarters of 1930 we made a special attempt to measure the crescent index of the population. We examined blood films of many persons, taking pains to secure a good representation of each age group, and adopting a standard number of microscopic fields to be devoted to the search for crescents. The results, already included in Table I, are here arranged by groups of months (Table IX):

TABLE IX

Comparison of the crescent incidence in a population during the second and third quarters of 1930

	July, August, and September	October, November, and December
Number examined	1,739	1,603
Number positive	1,074	898
Percentage positive	61·2	56·0
Among positives:		
Crescents, total incidence	16·8	16·0
Crescents, high infestations*	1·3	2·0

* One or more per 100 leucocytes

In the groups 1 to 11 months and 1 to 4 years both total crescents and heavier infestations of crescents were somewhat higher in the fourth quarter than in the third; while in the groups 5 to 8 years, 9 to 12 years, and 13 years and over, total crescents were higher in the third quarter, heavier infestations being nil in both quarters. On the whole, there was no striking difference in the crescent incidence of the two periods.

In concluding this Section we limit ourselves to the following generalisations :

1. The anopheline infection rate and, consequently, the malaria danger in this region are high in all months and especially so in August and September.

2. The September peak is probably correlated with the high anopheline density of June and July and the rapid decrease of *Anopheles* in August.

3. Infected *Anopheles* are widely distributed and their infection rate varies greatly in different localities.

4. Any valid comparison of the anopheline infection rates of different regions in Africa must be based on large numbers and take into consideration both locality and season.

IV. THE MALARIA PARASITE INDEX OF SPECIES OTHER THAN *A. COSTALIS*. LABORATORY INFECTION EXPERIMENTS. OUTDOOR BITING HABITS OF *ANOPHELES*

We found in the vicinity of Lagos the following species of *Anopheles*, in the larval or adult stage :

A. costalis Giles.
A. costalis, coastal race (? = var. *melas*, Theo.)
A. funestus Giles
A. hargreavesi Evans
A. moucheti var. *nigeriensis* Evans.
A. pharoensis Theo.
**A. mauritianus* Grp., type form.
A. mauritianus var. *ziemanni* Grünb.
A. mauritianus var. *paludis* Theo.
A. obscurus Grünb.
A. squamosus Theo.
A. nili Theo.

Specimens of all of the varieties listed here and of most of the species were sent to Miss A. M. Evans of the Liverpool School of Tropical Medicine, who kindly examined and identified them for us. For a description of specimens of the *marshalli* group collected by us, and of a melanic coastal race of *costalis*, referred to in the subsequent part of this report as '*A. costalis*, ? var. *melas*,' see Evans (1931).

In Table X we give a list of the *Anopheles* found in the adult stage, all in native houses of Southern Nigeria, the percentage incidence of species, the percentage found infected with malaria, and a notation on the presence of sporozoites in the salivary glands.

* One specimen only.

Besides the well-known carriers, *A. costalis* and *A. funestus*, we shall have to reckon in Southern Nigeria with *A. hargreavesi*, with *A. moucheti* var. *nigeriensis*, and with the black type of *A. costalis* common in the coast swamps near Lagos. All of these species occur in significant numbers and have a high infection rate. *A. nili*, which we found abundant and fairly highly infected in Liberia, was not common in any locality near Lagos. Schwetz (1929) found *A. moucheti* (*A. marshalli* var. *moucheti*) and *A. nili* infected in nature at Stanleyville, Belgian Congo.

TABLE X

Adult *Anopheles* collected in native houses of Southern Nigeria, Coast Region. Incidence and percentage infected with malaria parasites

Species	Number dissected	Incidence	Percentage malaria positive	Sporozoites in the salivary glands
A. costalis	15,144	96·3	12·0	6·6 per cent.
A. costalis (? var. *melas**)	—	—	—	Present
A. funestus†	70	0·4	15·7	12·8 per cent.
A. hargreavesi‡	92	0·6	7·6	5·4 per cent.
A. moucheti var. *nigeriensis*	87	0·5	2·3	1·1 per cent.
A. pharoensis	281	1·8	1·8	0·7 per cent.
A. obscurus	51	0·3	3·9	In none
A. mauritianus	2	0·01	0·0	—
TOTALS	15,727	99·91	11·7	

* The limits of this variety are not yet well defined. We have found infected in nature several specimens of *A. costalis* with very dark vestiture and a four-banded palps (? var. *melas*).

† Most of these specimens of *A. funestus* were collected in localities in which the infection rate of *A. costalis* was much higher than 12·0. In the Ibadan series of dissections (Table VIII) both *A. costalis* and *A. funestus* were collected in the same locality and during the same months. There the infection rate of *A. funestus* was lower than that of *A. costalis* in the totals and in nearly every month.

‡ About 136 specimens of *Anopheles*, species not surely identified, were also dissected. The most of these were very probably *A. hargreavesi*. If these be included with *A. hargreavesi* the percentage infected of that species becomes 3·1.

The importance of *A. pharoensis* and of *A. obscurus* in the transmission of malaria is more doubtful; possibly neither of them would be found infected in a region without such powerful allies as *A. costalis*, *A. funestus*, and *A. hargreavesi* to keep up a high game-

tocyte rate. *A. obscurus* is widespread in this region and breeds in localities difficult to treat by anti-mosquito measures.

Laboratory infection experiments.—Of late more emphasis has been placed on the value of laboratory infection of *Anopheles* because in such experiments we eliminate the possibility of confusion with bird, monkey, or other non-human malaria, the oocysts or sporozoites of which may rarely occur among mosquitos (*Anopheles* ?) collected in the open.

We have infected with human malaria the following species in the laboratory. In every case the parasite was malignant tertian.

A. costalis, see Tables XI and XII

A. costalis (?) var. *melas* (dark type with four-banded palps)

A. costalis (?) *melas*, bred in brackish water (dark type, three-banded palps)

A. hargreavesi

A. mauritianus (probably var. *ziemanni*)

Sporozoites were obtained in all except *A. mauritianus*, in which only two poorly-developed oocysts were found in one individual.

In several tests we got negative results with *A. mauritianus* (both varieties) when our *A. costalis* controls were positive. Our results agree with those of McGregor in Mauritius, who considered this species of little sanitary importance.

We got negative results with *A. obscurus* when *A. costalis* controls were positive, but the batch was small and the results inconclusive. This species did not bite well nor survive long in captivity.

We give in more detail (Tables XI and XII) certain results obtained in laboratory infections of *A. costalis* with malignant tertian. In these the crescent rate and the degree of infection of the mosquito are compared. In both tables only specimens of *A. costalis* which had fed but once are included, and in Table XI only such as had become fully engorged with blood.

Table XI contains the results obtained with about twelve different carriers and includes also repeated ' bitings ' on the same carrier. The results obtained with a single carrier (' Taiwo ') are given in Table XII. These are included in Table XI, but are repeated here to show the long persistence of viable crescents in the

TABLE XI

Relation of crescent percentage among children to the infection rate of *A. costalis*

Percentage of crescents	Number of *Anopheles* dissected	Percentage *Anopheles* infected	Average number of oocysts per mid-gut among positives
0·2	9	0·0	0·0
0·47	10	10·0	1·0
0·47	2	0·0	0·0
0·5	5	0·0	0·0
0·7	3	0·0	0·0
0·8	16	50·0	1·4
TOTAL 0·2 to 0·8	45	20·0	1·3
1·4	3	33·3	1·0
1·6	3	0·0	0·0
1·6	3	66·7	31·5
1·6	2	100·0	1·0
1·6	8	37·5	1·3
1·9	4	75·0	17·3
1·9	4	50·0	6·0
TOTAL 1·4 to 1·9	27	48·1	10·3
2·1	5	100·0	4·8
3·9	1	100·0	2·0
4·1	1	100·0	5·0
5·1	2	100·0	66·0
5·8	1	100·0	4·0
5·9	10	100·0	151·9
TOTAL 2·1 to 5·9	20	100·0	84·2
6·6	7	0·0	0·0
7·7	10	40·0	3·7
11·3	11	90·9	189·0
TOTAL 6·6 to 11·3	28	50·0	136·1

patient, an infant of 3 months. On several occasions during the experiment the mother of ' Taiwo ' was given quinine to administer to the baby. How faithfully the medicine was given is not known, but it is highly probable that the patient got most of it. It is well-known that quinine does not render crescents non-viable; that it promotes crescent formation is believed by some authors, but this has not been satisfactorily proved.

TABLE XII.

Relation of the crescent percentage of patient ' Taiwo ' to the infection rate of *A. costalis*.

Date of feeding *Anopheles*	Percentage crescents	Approximate percentage female crescents	Number *Anopheles* dissected	Percentage *Anopheles* infected	Average number oocysts per mid-gut among positive
1930					
September 27	11·3	58	11	90·9	189·0
*September 30	8·1	71	5	60·0	22·0
October 4	5·9	60	10	100·0	151·9
October 8	5·1	76	2	100·0	66·0
October 15	1·6	66	3	66·7	31·0
October 18	1·6	75	2	100·0	1·0
October 29	0·47	62	2	1·0	0·0
November 2	0·1	...	1	0·0	0·0

* *Anopheles* only partly engorged.

The correlation of crescent rate and anopheline infection, as shown in these tables, is not a high one, possibly because of the varying maturity of crescents or of the disparity of sexes among them; but the correlation is apparently high enough to show that heavy crescent carriers (in Southern Nigeria infants and very young children) are the ones most likely to transmit malignant tertian malaria.

Outdoor biting habits of Anopheles.—We owe the following data on the outdoor biting habits of *Anopheles* to Dr. J. A. Kerr, of the Yellow Fever Commission, Yaba, who kindly put his results at our disposal. Dr. Kerr used as mosquito ' baits ' native Africans stationed outdoors at night, who caught mosquitos as they attempted

to bite. Some baits were kept out all night, but the most of them only during the hours 6.30 p.m. to 10.0 p.m. These observations were made during the period, September to December, inclusive, 1930. With very few exceptions all *Anopheles* caught were females. The numbers caught and percentage incidence of species are shown in Table XIII.

TABLE XIII.

Species, numbers, and percentage incidence of *Anopheles* caught outdoors, Coast Region.

Species	Number caught	Incidence
A. mauritianus var. *paludis**	292	56·5
*A. hargreavesi**	160	30·9
A. costalis	46	8·9
A. obscurus	13	2·5
A. pharoensis	6	1·2
Total	517	100·0

* At the time this work was done the limits of certain species were not definitely known. An insignificant percentage of *A. mauritianus* var. *ziemanni* may have been included with the *paludis*, and of *A. moucheti* var. *nigeriensis* with *hargreavesi*.

Comparing Tables X and XIII, we note that *A. mauritianus* bites freely in the open but is very rarely found in dwellings; while *A. costalis* rarely bites outdoors but constitutes the principal species found in houses. *A. hargreavesi* occupies an intermediate position.

A few notes on collections made by ourselves outside of dwellings may be of interest here:

August 4 to August 25, 1930, in a garage: sixteen collected, all *A. costalis*; two infected, both with sporozoites in the salivary glands.

July 16 to October 1, 1930, on screens outside of the house occupied by one of us (M. A. Barber): twenty-five collected, all *A. costalis*; two infected, one with sporozoites in the salivary glands. Noteworthy was the large percentage of empty, non-fertilised females found in the latter collection. Apparently many of these had emerged the night before collection.

Evidently malaria is not strictly a house infection in this region. We believe that the distinction between 'domestic' and 'wild' species as regards the transmission of malaria has received undue emphasis in the literature.

V. THE DISPERSION OF ADULT *A. COSTALIS*

The range of dispersion of *Anopheles* is of the greatest importance to sanitarians everywhere; it is especially so in this region where the population is dense and so highly infested with malaria that *Anopheles* may frequently be infected when they leave the neighbourhood of their breeding place.

In a study of the dispersion range of *A. costalis* we made observations in three localities.

1. *Yaba.*—During the period, February 11 to May 5, 1930, we attempted to do away with all *A. costalis* breeding in an area around the West African Yellow Fever compound at Yaba. We personally did the scouting for breeding places, the application of larvicides (Paris green and crude oil), and the checking up on the results of the treatments. We examined many water pots in and about dwellings near Yaba and found no anopheline larvae in them. We also examined many wells, using the Bentley apparatus for collecting larvae, and found breeding in only two wells, both very wide at the top and essentially pits rather than wells. Nearly all of the wells in this vicinity are covered or narrow at the top. We can state that in all probability not a single breeding place of *Anopheles* within a radius of half a mile escaped us, except one well-pit that was being extensively used by the people and had a negligible number of *A. costalis* larvae in it.

Within the mile radius there are extensive wooded or brushy swamps productive of *A. mauritianus, A. hargreavesi,* and *A. obscurus.* We made many surveys in these but never found *A. costalis.* So we can state with reasonable assurance that within a mile radius from the Yellow Fever Compound the production of *A. costalis* was negligible. We were materially assisted by dry weather which did away with most of the shallow breeding places. The greater part of the *costalis*-producing water in the treated area consisted of shallow ponds, some of which persisted throughout the dry season.

We extended the treatment of ponds to a point about a mile and a half distant in the direction from which the prevailing winds blew.

We measured the results by collections of adult *A. costalis* in certain houses occupied by native employees of the commission. The numbers of this species declined somewhat after we began our 'control,' but remained fairly high, higher than during the dry period, December, 1930, to January, 1931, during which no larvicidal work was done. Neither the room density nor the infection rate of *A. costalis* showed a decline of material importance.

It is probable that the *A. costalis* found in the Yellow Fever Compound were coming from a large coastal swamp beginning a little over a mile from the houses in which our collections were made. This seems the more probable since collections in Yaba during dry seasons contain many of the dark form of *A. costalis* (described in Section VI), which in this region seem to be bred almost exclusively in coastal swamps.

We made regular collections of *A. costalis* at the police barracks of Yaba, situated near the margin of the coastal swamp, and in Idioro, a village situated about a quarter of a mile farther from the swamp than our collecting place in the Yellow Fever Compound. During the drier months of the year the density of *A. costalis* was consistently highest at the police barracks and lowest at Idioro, suggesting a diminution of anopheline density as the dispersion range became greater.

In the course of our experiments we found that the application of Paris green had to be repeated every five days. We used this larvicide freely and on certain occasions made very careful inspections to assure ourselves that all larvae had been killed. The growth rate of larvae of *A. costalis* is so rapid, especially during the hot season in ponds open to the sun, that eggs, which are not destroyed by this larvicide, may hatch and reach the pupal stage within five days. The application of oil had to be repeated at intervals of only about eight days, an interval which might have to be shortened during a period of rains sufficiently heavy to disperse the oil film.

2. *Otta Town.*—Otta Town is a large town situated in a hilly region about 25 miles from Lagos. It is surrounded by forest interspersed with small farms. Within the town the ground water level is so low that wells are impracticable, and during the dry season

water has to be carried from a stream about half a mile away. The density of *A. costalis* is very low, but the infection rate exceedingly high—40 per cent., the average of 202 dissected. The collections for these dissections extended over a period of ten successive months, and included fifteen different batches.

We surveyed the forests around this town for a mile or more in every direction. We found only one breeding place of *Anopheles*, a permanent stream with swampy margins, situated about half a mile from the town. This, however, contained only larvae of *A. hargreavesi*, *A. obscurus*, and *A. mauritianus*. We have never found *A. costalis* in this sort of breeding place at Otta Town or elsewhere. We also examined a large number of water pots placed in the open. We found many culicines, but not a single anopheline larva in them. The only permanent, dry-season, breeding place of *costalis* we could find consisted of small pools formed in the laterite bed of a ravine situated at the edge of the town.

We treated these pools with oil at about eight-day intervals during a period of nearly 6 weeks, December 18, 1930, to February 1, 1931, and before each treatment we inspected the water for anopheline larvae.

We found the following range of anopheline density (average per room) in the town. The same group of houses was inspected before and after the treatment of the pools.

Month	Number dissected	Rate per room
November	7	0·4
December, first half	9	2·3
December, second half	8	0·6
January, first half	2	0·1
January, second half	5	0·1

This index is based on small numbers; only one visit was made in November, three in December, and four in January. The sporozoite rate of the *A. costalis* dissected rapidly increased; in the collection of January 23 both of two dissected contained sporozoites in the salivary glands.

Not much can be concluded on the basis of such small figures,

but it is probable that the ravine pools were the breeding place of the *A. costalis* found in dwellings, and that none was coming in from the swampy stream described above nor any significant percentage from water pots. The few females appearing in the later collections were probably survivors of an earlier period. This work was done in the dry season. During periods of continuous rains probably many breeding places would occur within the town.

We examined blood specimens of seventy school children in Otta Town and found a malarial parasite rate of 92·9.

3. *Lagos.*—As regards anopheline dispersion, the portion of Lagos of particular interest is that situated to the north-west and projecting into the lagoon. (See map, page 475.) This district is protected from anopheline invasion by a barrier of nearly half a mile of water on three sides and a mile or more of densely populated city on the fourth. The lagoon itself is here unsuited for anopheline breeding. The soil of the district is sandy and its natural drainage good. The streets are visited regularly by an oiling squad.

In every one of five surveys for anopheline breeding places, made during both wet and dry seasons, we found anopheline larvae or pupae. The breeding places were few and usually consisted of very small collections of water formed by seepage or rains and retained in the pervious soil by silt or layers of algae. They usually occurred in compounds and were only twice found in the streets. All *Anopheles* bred out from these collections were *A. costalis*.

The anopheline adult density of this district was low. In the course of seventeen surveys over the period, October, 1929, to January, 1931, inclusive, we inspected 305 rooms and found an index of only 0·20 per room. In every survey the African collector was accompanied by one of us who entered the rooms with him. All *Anopheles* found were *A. costalis* and all were females except one. Among fifty-six dissected the total malaria infection rate was 19·8, and the sporozoite rate, 8·9 per cent.

The source of the adult *Anopheles* found in this district is of chief interest. It seemed improbable that the very few breeding places found could supply the numbers of *Anopheles* caught. The anopheline density index was very low, but there are many hundreds of rooms in this district and no search can detect every anopheline in an African house. In our surveys we compared two localities: (1) Marina

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November	7	0·4
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Street, situated at the margin of the lagoon; and (2) the Ereko neighbourhood situated well within the city. In several surveys collections were made in each locality at the same or nearly the same time. The density index was almost always higher in Marina Street than in Ereko. In the totals, 142 rooms on the Marina gave an index of 0·31 and 163 rooms at Ereko, 0·09. It seemed most probable that many of the adult *A. costalis* found in that district were coming across the lagoon from extensive swamps situated about half a mile away.

In view of the low anopheline adult index of this part of Lagos the parasite index of children residing there is of interest. Ereko Wesleyan School, eighty-one children 4 to 8 years old, gave an index of 96·2 per cent. at one examination and almost exactly the same index at an examination made a month later. Moslem Religious School, 188 children 4 to 15 years of age, gave an index of 85·6, and St. Paul's Breadfruit, 437 children, 4 to 18 years old, 78·2. Among children picked up at random in the Ereko neighbourhood, four, one year of age, were all positive; and of seven, 2 years of age, five were positive. The parasite index of 1,108 infants 3 or 4 months old of Lagos was 17·1, but this number includes infants of all parts of the city and the index is not strictly valid for the district in question. Taking all evidence, however, it is probable that few children of this part of Lagos escape infection during the first year of life.

Summary of Section V.

1. *A. costalis* will disperse at least half a mile from a large breeding place and probably much farther.

2. A very low adult density index in this species seems to be adequate to keep up a high malaria rate.

3. No considerable proportion of *A. costalis* are bred in wells or water pots.

It is to be emphasised that these conclusions are valid only for this region, where suitable breeding water and blood supply are almost everywhere available. In a very dry climate this species may seek wells or any water obtainable and, in localities where breeding places are isolated, may make long flights to dwellings. Symes (1930) reports a flight of *A. costalis* of over three miles in Kenya.

VI. THE BIOLOGY AND BREEDING PLACES OF *ANOPHELES*. ANTILARVAL MEASURES

A. costalis.—Our observations in the vicinity of Lagos indicate that larvae of *A. costalis* are very adaptable to various breeding places. They occur in fresh or brackish water (January 22, 1930, near Apapa, 66·5 per cent. sea water). They thrive in the sun where the water-surface temperature at mid-day reaches 104° F., or in the shade (pool near Inupe thoroughly shaded by palms and a broad-leaved tree). They develop in very shallow rain pools or at the margins of a large lagoon. They occur in canoes and occasionally in pots placed in the open. We do not believe, however, that water pots, even when set out of doors, constitute an important source of this species in the regions we have studied. (See Section V.)

Certain types of water, apparently favourable, do not commonly harbour larvae of *A. costalis*. They are uncommon or lacking in the large wooded or semi-wooded swamps common in this region even when the water is partly exposed to the sun. A large fresh water pond near Ijora Village (' Lily Pond '), exposed to the sun and full of aquatic vegetation, is curiously free from *Anopheles*, at least the eastern part of it. We have surveyed it carefully during the following months: January, February, April, May, July, August, and September, and have found only culicines. Eggs of *A. costalis* were placed in water freshly transported from this pond to a tank near the laboratory. Adults, normal and eager to bite, formed in eight days from the time of oviposition. We cannot explain the freedom of this lake from *Anopheles*. The presence of numerous culicines, including the surface-feeding Uranotaenia, would indicate that it is not due to natural enemies, and the tank experiment demonstrates that the water itself is favourable to the development of larvae of *Anopheles*.

We have found that *A. costalis* will develop from egg to adult in 7 or 8 days, probably 7 days in ponds or shallow pools exposed to the sun. We have watched their development in newly formed pools and in the tank just described. Females will bite freely during the forenoon following emergence. We have proved by our experiments on crescent carriers that sporozoites may be found in the salivary glands 9 days after feeding. So that the minimum time between the hatching of eggs and the production of sporozoites is only 16 or 17 days. In a large mosquito population, however, it is probable

that nearly a month would elapse before new broods show a measurable increase in the sporozoite rate.

Females of *A. costalis* have a voracious but erratic appetite, as shown by an experiment described in Table XIV. A batch of six females were placed in separate cages and given a daily opportunity of feeding on a human subject. It is noteworthy that five out of the six, all taken at random, survived 10 days. In this table 'X' means that the mosquito bit and became engorged; 'O' that it refused to bite.

TABLE XIV

Feeding experiment on *A. costalis*.

Mosquito check number	Days after emergence											
	1	2	3	4	5	6	7	8	9	10	11	12
1	O	X	O	X	O	X	O	O	O	X	O	X
2	X	O	O	X	Died	...	...	...	...	...	...	...
3	X	X	O	O	O	X	O	O	X	O	O	X
4	X	X	X	O	O	O	O	O	O	X	Died	...
5	X	O	X	X	X	O	X	O	X	O	X	O
6	X	O	O	X	O	O	X	O	O	X	O	Lost

A. costalis, coastal race (? = var. *melas*).—A form of *A. costalis* with very dark larvae and dark adults, some of which exhibit the four-banded proboscis, occurs plentifully in the large coastal swamps surrounding Lagos. The limits of this form are not well defined; but that it is biologically different from the type of *A. costalis* common inland is indicated by certain experiments which we conducted in the laboratory.

Eggs from adults of *A. costalis* collected near the coastal swamps developed normally when placed in either fresh or brackish water, while those from adults collected far inland (Ibadan) failed to develop beyond very small larvae in the same type of brackish water. Controls of inland source developed normally in fresh water. We repeated this experiment several times, always with the same result.

The swamps in which the (?) *melas* type breeds are fresh during

the greater part of the rainy season and brackish (about 46 per cent. sea water) during the dry. They are scarcely above sea-level and are commonly covered by a coarse grass, *Paspalum vaginatum* Swartz. During both fresh and brackish water seasons, the water of these swamps in many places overlies a very black, thin mud not commonly found inland. We have only occasionally found black larvae of *A. costalis* far inland and have never bred out typical *melas* from them.

A. funestus.—Streams of the type commonly harbouring *A. funestus* are rare in the coast region, and the species is relatively uncommon there. We found their larvae most plentifully in a pond open to the sun and abundantly supplied with dead grass. They also occurred more rarely in partially wooded swamps, in pools covered with *Pistia*, in muddy ditches, and in a large clear river (Ogun River at Ishiri).

A. hargreavesi was most plentiful in certain Pistia-covered pools, and occurred more sparingly in grassy swamps.

A. moucheti var. *nigeriensis* occurred sparingly in partly wooded swamps and in the Ogun River.

A. pharoensis was common in ponds, especially those with grassy margins. It also occurred in the coastal swamps during the fresh-water season, but tended to die out as the water became brackish.

A. mauritianus var. *ziemanni* occurred very plentifully in certain shallow grassy ponds open to the sun. We did not find it elsewhere. The var. *paludis* was much more widespread, and was especially common in ponds and rivers well supplied with living aquatic vegetation.

A. obscurus was found in ponds and swamps, both open and wooded, but seemed to prefer more shaded waters. Larvae usually occurred in small numbers, but were sometimes plentiful among the dead fronds of ferns overhanging water in floating debris in wooded swamps and in cocoa-tree nurseries, which are usually planted in wet, shaded ground. They persisted in the coastal swamps long after the water became brackish, but tended to die out when the salt content of the water increased.

A. squamosus occurred sparingly in ponds, often associated with *A. costalis*.

A. nili, a common stream breeder in some regions, was found here in only one locality—among aquatic plants in the Ogun River near Ishiri.

It appeared from our surveys that almost no type of water, aside from the sea, could be disregarded by the sanitarian in this region. The large wooded swamps, containing fresh water and covering many hundreds of acres of the coastal plain, would seem to be the least dangerous because they rarely harbour *A. costalis* or *A. funestus*.

As the dry season advances the proportion of adult *A. costalis* found indoors increases, probably because the water in ponds tends to retreat from the grassy margins and become less favourable to the larvae of any other species.

Anti-larval measures.—In regions where *A. costalis* abounds anti-larval measures meet with special difficulties. Larvae are adapted to a great variety of breeding places, and their development is very rapid, enabling them to take advantage of very temporary collections of water. Adults are domestic in their habits, very susceptible to malarial infection, and capable of wide dispersion.

For many years to come any extensive anti-larval measures will probably have to be confined to the protection of towns or other limited areas. In some cities of West Africa effective work has already been done, resulting in a diminution of the mosquito nuisance and of the danger of malarial infection. These results have been obtained by more permanent measures such as drainage and filling and, to a less extent, by the use of larvicides. We believe that these more permanent measures are the ones best suited for conditions in West Africa. Larvicides will always be necessary no matter how thorough the drainage or filling. Small pools will form during the rainy season especially along human or animal footpaths and these will require continuous inspection and treatment. But we doubt if any larvicidal ' campaign ' can alone cope with conditions found in West Africa, unless, perhaps, in localities especially favoured for such work. Otta Town, described in another part of this section, would be a good locality for experiment with anti-larval measures.

Whatever the measures employed one would not expect in Nigeria the early results reported of anti-larval measures in less difficult regions. The present rate of human infection is so high here, and

the anopheline density necessary to maintain this rate so low, that malaria is likely to persist for many years among a people little protected against infection.

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APPENDIX

A STATISTICAL ANALYSIS OF THE INTENSITY OF MALARIA INFESTATION

BY

PERSIS PUTNAM

At Dr. Barber's request I have made a statistical analysis of the degree of malaria infestation among native Nigerians in various age groups, a brief discussion of which follows.

The results of blood examinations were classified according to the number of parasites per thick film field. As many as 150 fields were examined before a specimen was pronounced negative. Films showing fewer than one parasite per field were classified into two groups, the first in which one parasite was found in 24 fields or fewer, and the other in which one parasite was found in from 25 to 150 fields. In the following analysis the latter group was not included although, strictly speaking, it must be considered an infected one.

Ages of individuals examined extended from 1 to 2 months through approximately 36 years. The number included in the 1 to 2 months' group was too small to consider separately and has been omitted.

The data analysed are given in the following table according to age and degree of infestation. The observations are highly concentrated in the lower density groups. This is true of each age class. At earlier ages the data are scattered over a considerable range of increasing intensity of infestation, but this tendency is less marked as age advances.

The asymmetrical form of these distributions raises the question of what centering constant to use to bring out fairly the changes in intensity with age. To ascertain roughly the amount of asymmetry present, the arithmetic mean and median were calculated and the difference between them determined for the distribution at all ages (column 13 of the table). The results follow :

Malaria Parasites per Field, all ages :

Arithmetic mean	$= 8{\cdot}23 \pm {\cdot}54$
Arithmetic median	$= 1{\cdot}24 \pm {\cdot}68$
Difference	$= 6{\cdot}99 \pm {\cdot}87$

The median indicates the amount of infestation to be found at the midpoint of the distribution. One half of the 3,080 observations lie on either side of this point. The selected measure of intensity should lie somewhere near the centre of the distribution it describes. Here the mean value has been raised above the centre due to the weighting of the extremely high intensity of a few observations. The difference between it and the median is 8 times its probable error.

Intensity of malaria infestation among persons carrying parasites in fewer than 25 fields, by age

Parasites per thick film field	Age of infected persons											
	3 months	4 months	5 to 11 months	1 year	2 years	3 years	4 years	5 to 8 years	9 to 12 years	13 to 15 years	Over 15 years	All ages
1 in 24 to 1 per field ...	63	11	20	12	9	15	32	321	351	203	386	1,423
1 per field ...	24	4	13	5	3	15	26	186	118	51	46	491
2 to 5	38	12	26	13	14	28	43	244	86	43	30	577
6 to 10... ...	20	8	21	12	11	16	19	81	29	11	12	240
11 to 20... ...	21	8	14	8	14	12	7	54	16	3	3	160
21 to 50... ...	26	4	9	9	6	9	6	28	5	3	3	108
51 to 100 ...	12	4	9	3	6	1	2	9	0	1	0	47
101 to 200 ...	8	1	4	2	3	1	2	1	0	0	0	22
201 to 500 ...	6	0	0	1	0	0	0	0	0	0	0	7
501 to 1000 ...	1	0	2	2	0	0	0	0	0	0	0	5
TOTAL ...	219	52	118	67	66	97	137	924	605	315	480	3,080
Mean Parasites per field (geometric) ...	3·40 ±·34	3·84 ±·52	4·68 ±·60	5·52 ±·94	6·38 ±·93	3·31 ±·35	2·10 ±·19	1·34 ±·07	0·61 ±·02	0·51 ±·03	0·34 ±·01	1·07 ±·01
Total examined ...	1,200	205	243	80	78	110	156	1,104	919	638	2,139	6,872
Total infected ...	270	61	140	72	73	107	145	1,033	796	486	1,036	4,219
Per cent. infected	22·5	29·8	57·6	90·0	93·6	97·3	92·9	93·6	86·6	76·2	48·4	61·4

If the geometric mean and median are computed, centering values are obtained that are more nearly identical. For this same distribution these are :

Malaria Parasites per Field, all ages :

Geometric mean = 1·07 ± ·02
Geometric median = 1·18 ± ·03
Difference = ·11 ± ·04

The geometric mean is lower than the arithmetic mean because less weight is given the extreme points in the distribution. The median in both cases is about the same, but now the difference between mean and median is not quite 3 times its probable error.

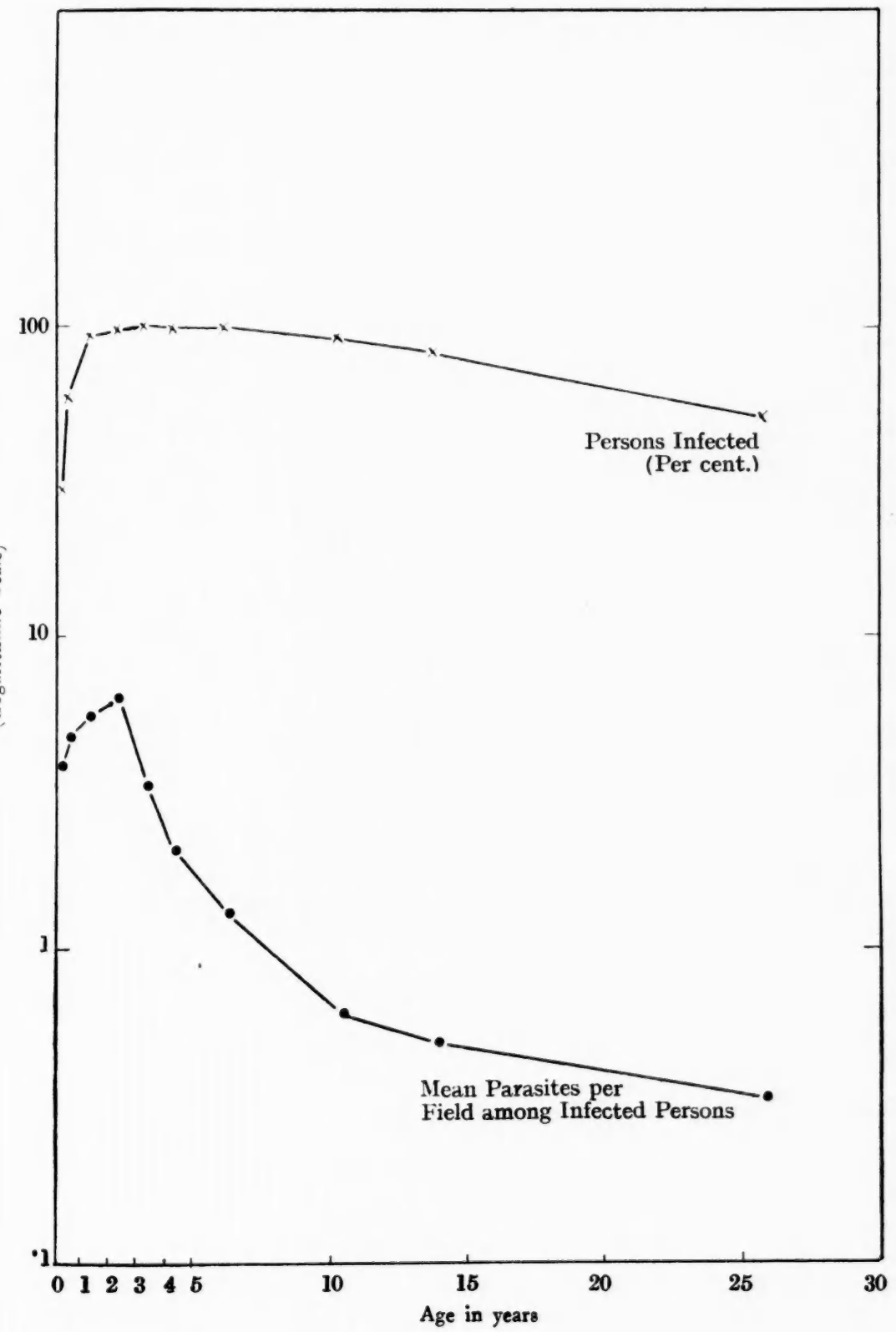
To bring the intensity index closer to the centre of the distributions, geometric means for the observations in each age group have been computed. In cases where the resulting mean and median were compared the differences were insignificant. The results are given in the table and plotted on the following diagram (fig. 4). To compute these constants logarithms were used to set the limits and mid-points of the intensity classes and the interval between them. The logarithm of the geometric mean is the arithmetic mean of the logarithms of the observations.

In plotting these means a geometric scale has been used to bring out the percentage changes with age. The graph shows a rapid rise from 3·40 parasites per field at the age of 3 months, to 6·38 at the age of 2 years. Intensity then falls sharply and continues to decrease at older ages. Although the number of observations in some of the age groups was small, the means are significant, and the curve has a definite form.

For purposes of comparison, the malaria incidence rate has been added to table and graph. The infected individuals upon which this rate is based include the low intensity group omitted in the foregoing analysis. The interesting feature of this comparison is that incidence rises with age much as intensity does but remains at its maximum longer and does not drop so rapidly.

FIGURE 4

Comparison of malaria incidence with intensity of infestation by age



METEOROLOGICAL DATA

Ibadan.

Average for period 1920-1929, inclusive.

Month	Mean temperature	Absolute maximum	Absolute minimum	Mean humidity	Rainfall
January	82·2°	99·8°	62·6°	76·6%	0·43″
February	84·6°	102·4°	63·6°	84·9%	0·89″
March	84·9°	103·1°	64·5°	84·7%	2·65″
April	82·5°	99·5°	64·3°	86·4%	4·95″
May	80·2°	97·6°	63·5°	88·4%	5·90″
June	79·1°	94·5°	65·6°	85·8%	8·39″
July	76·8°	92·3°	65·4°	91·7%	5·89″
August	76·3°	91·1°	64·2°	90·3%	2·77″
September	77·5°	92·8°	65·5°	90·8%	7·38″
October	80·1°	95·5°	65·6°	91·1%	5·74″
November	81·6°	96·4°	67·0°	87·0%	2·09″
December	80·7°	97·3°	64·3°	85·4%	·22″

Lagos.

Average for period 1920-1929, inclusive.

Month	Mean temperature	Absolute maximum	Absolute minimum	Mean humidity	Rainfall
January	81·6°	90·4°	67·8°	77·6%	0·99″
February	82·8°	92·0°	71·5°	79·6%	1·27″
March	83·4°	92·7°	70·2°	78·3%	3·86″
April	82·7°	91·4°	71·8°	77·9%	6·90″
May	81·0°	91·8°	71·6°	79·9%	11·63″
June	79·7°	87·9°	70·6°	83·2%	17·37″
July	77·9°	85·5°	70·7°	83·5%	9·84″
August	77·7°	84·5°	70·3°	81·1%	4·67″
September	78·9°	85·3°	71·0°	81·7%	5·57″
October	79·6°	87·7°	70·5°	81·3%	8·39″
November	81·5°	89·9°	73·3°	79·5%	2·86″
December	81·8°	90·6°	70·4°	78·9%	1·26″

Lagos, 1929.

Month	Rainfall in inches	Mean temperature	Relative humidity
January	·02″	80·8°	71%
February	1·46″	82·7°	75%
March	1·73″	83·5°	77%
April	1·04″	82·0°	78%
May	11·34″	79·4°	78%
June	24·79″	78·4°	85%
July	19·93″	77·5°	86%
August	·81″	78·0°	79%
September	3·11″	78·7°	79%
October	6·03″	78·7°	79%
November	4·10″	80·7°	79%
December	6·02″	79·4°	74%
Total	86·38″	959·8°	940%
Average	...	80·0°	78·3%

Lagos, 1930.

Month	Mean temperature	Absolute maximum	Absolute minimum	Mean humidity	Rainfall
January	78·9°	89°	69°	79·8%	1·38″
February	84·5	91°	72°	77·7%	2·21″
March	83·8°	92°	72°	76·8%	3·27″
April	82·5°	92°	71°	77·4%	5·01″
May	82·1°	90°	72°	77·8%	8·61″
June	79·8°	88°	70°	82·7%	13·28″
July	78·4°	88°	73°	84·7%	18·40″
August	78·4°	86°	70°	79·6%	·66″
September	79·4°	86°	72°	81·2%	2·67″
October	80·4°	88°	71°	82·0%	13·06″
November	82·2°	92°	71°	77·3%	1·88″
December	83·1°	91°	72°	79·5%	1·67″
Total	973·5°	1,073°	855°	956·5%	72·10″
Average	81·1°	89·4°	71·3°	79·7%	...

Month	Ibadan, 1930.											
	Jan.	Feb.	Mar.	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
Mean maximum ...	90·3°	92·8°	92·0°	84·1°	87·7°	83·9°	82·4°	80·5°	82·6°	82·4°	88·7°	89·4°
Mean minimum ...	68·6°	74·4°	75·2°	70·3°	73·3°	70·6°	71·5°	70·9°	71·7°	71·3°	72·8°	74·6°
Mean humidity ...	73·0%	77·0%	81·0%	80·0%	80·0%	85·4%	87·4%	87·4%	82·0%	87·6%	83·0%	84·8%
Total rainfall ...	Nil	0·15″	5·86″	3·54″	5·96″	10·09″	11·52″	1·56″	7·06″	6·60″	0·29″	0·64″
Mean solar maximum ...	143·6°	144·3°	147·0°	146·8°	149·0°	139·0°	135·7°	134·3°	116·8°	136·5°	126·4°	107·9°

THE TRANSMISSION OF HUMAN AND BOVINE ONCHOCERCIASIS

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HISTORICAL REVIEW OF *O. VOLVULUS* Leuckart, 1893

Brumpt (1903) suggested tsetse flies, tabanids or simuliids as possible carriers of *O. volvulus*, but he soon recognised that tsetse flies, at least, were impossible; he further pointed out that the localization of the parasite along rivers seemed to exclude mosquitos.

Leiper (1913) in Nigeria, injected a large number of living embryos of *O. volvulus* subcutaneously and intraperitoneally into a monkey, but none appeared in its blood. He fed specimens of *Stomoxys nigra* and *S. calcitrans* upon the juice of tumours containing many embryos. On dissection at later dates, no evidence of infection was noticed in the flies, although embryos were found in the stomach contents.

Rodhain and Van den Branden (1916) made experiments with *Stegomyia fasciata* and *Cimex rotundatus*, by feeding these on a meal of blood which contained living embryos of *O. volvulus* from a cyst. Four days later the insects were killed and examined, but no developmental stages were found.

Calderón (1917) stated that Robles came across a disease in certain parts of Guatemala, associated with the presence of tumours on the head, containing a filaria, like *O. volvulus*. Investigation showed that two species of *Simulium* were present in the infested zones, and that where these insects were most numerous, there also were most cases of the disease. Calderón (1920) tried lice, mosquitos, and various flies, to see if they function as intermediate hosts for *O. caecutiens*, as this South American Onchocerca was named, but none yielded a positive result. In his opinion the

transmitter must be a nocturnal biting insect, as all the tumours occurred on the head, and he strongly suspected one of the *Culex* mosquitos.

Macfie and Corson (1922) in the Gold Coast, made feeding experiments with *Glossina palpalis*, *G. longipalpis*, and *Pediculus humanus corporis* with negative results. They also dissected some *P. humanus corporis* for the presence of *O. volvulus* embryos without success.

Fülleborn (1923) reported the presence of *O. caecutiens* and *Simulium* in Mexico.

Blanchard and Laigret (1923) finding embryos of *O. volvulus* in the skin, fed *O. moubata*, *Cimex rotundatus*, a species of *Simulium*, *Auchmeromyia luteola*, and leeches upon African natives infected with *O. volvulus*. In most of their experiments a few embryos were found in the ticks, flies and leeches after the infecting feed, but as a rule they disappeared soon afterwards. In the tick, however, they lived up to 12 days. In no case was any definite development of the embryos observed. They experimented with only twenty *Simulium*, none of which survived until the next day.

Blacklock (1926) noticing that many individuals whose skin was heavily infected with *O. volvulus* had no larvae in their blood, thought it probable that if any blood-sucking arthropod was the vector, it would be one which had to rasp and tear the skin in order to reach the blood, thus dislodging the larvae, which would then be ingested with the blood. He found that the lesions made in the skin by *Auchmeromyia luteola* (Congo floor maggot), and *S. damnosum* were of this nature; the latter was abundant beside the small streams.

Numerous individuals of these two species were examined for the presence of *O. volvulus* in the gut with negative results in 1923. The investigation was resumed in 1925 on a very large scale with a successful result. He proved the development of the *O. volvulus* embryos in *Simulium damnosum*, and followed the developmental stages in the gut, thorax, head and ultimately in the probocis of that insect. In a later paper he suggested that *O. caecutiens* might be transmitted by *Simulium*.

Connal (1926) in Nigeria dissected *S. damnosum*, collected from different districts, for the presence of filaria, with negative results.

The Harvard African Expedition (1930) collected *S. damnosum* in Liberia when biting, and found embryos in the gut and later in

the thorax of the flies. They found the filaria embryos in *S. damnosum* in localities where *Onchocerca* infection was present.

Hoffman (1930) in Mexico reported the normal development of *O. caecutiens* in *Simulium mooseri*. Strong, 1931, confirmed this, and proved that *Simulium* was a carrier.

O. GIBSONI (Cleland and Johnston, 1910)

Cleland and Johnston (1909) enumerated the following possible methods of transmission, with the points for and against each, viz. :—

(1) By insects, like mosquitos and biting flies or ticks. (2) By a life cycle like that of the guinea-worm ; the embryos pierce the skin and reach water, where they are ingested by a host like cyclops. (3) By direct transmission.

Gilruth and Sweet (1911) finding that larvae sown on grass and soil rapidly died, dismissed this as a possible method. They injected living embryos subcutaneously and intramuscularly into young cattle, with negative results on subsequent *post mortem* examination. They excluded the agency of native dogs, and wild pigs and also of ticks, leeches, and biting flies, but expressed the belief that lice offer the most hopeful possibility. These authors (1912) transplanted a nodule containing living embryos subcutaneously into a calf, but infection did not occur. They spread soil from under infected cattle, in a pen where a non-infected calf was kept ; this was killed seven months later, but no evidence of infection was discovered.

Lice failed to convey the infection, as did direct contact of infected and non-infected cattle screened in pens.

They concluded that direct contact or intermediation of soil were not responsible for transmission, nor could lice normal to cattle act as vectors. They also excluded ticks and commented that every probability pointed to the intermediate host being a fly.

Cleland (1912) found three infected calves which were born and bred in Milson Island ; their mothers were not infected so that the disease was not acquired transplacentally ; ticks and leeches were absent on the island. He also dismissed direct infection by water or aquatic animals as the district was dry, and, having enumerated as possible hosts *Stomoxys calcitrans*, Mallophaga, Tabanidae, the sandfly, *Culicoides molustus*, lice and mosquitos, he attached most importance to *Stomoxys*.

Breinl (1913) observed that the larvae could pierce the intact skin of the host and find their way to the exterior, although this occurred only in a few cases out of a large number of experiments he supported the theory of water borne infection.

Nicoll (1914) carried out over thirty experiments to control and confirm the above observations of Breinl and in no case did he notice any escape of larvae from the tumours through the skin to the exterior. He also argued against the water borne theory, that the larvae are not adapted for independent existence in water, and that they do not live in it for more than two days. Insect borne infection, he said, presented the greatest measure of possibility.

Cleland (1914) stated that *Stomoxys calcitrans* is uniformly distributed all over Australia, and attacks cattle and horses in the lower part of the legs and brisket. These situations are suggestive of embryos being injected by some insect host. He fed a large number of *S. calcitrans* on fresh worm nests containing embryos and found that these lived in the fly up to three days, but no development occurred. Further, flies were caught in endemic areas and dissected with negative results. Feeding experiments with *Musca domestica* and *M. vetustissima* showed that the embryos were dead in these flies one in two days after the feed. Mosquitos ingested the embryos, but again these were found dead. An experiment was made to infect a calf by means of *S. calcitrans* some of which had fed on cattle harbouring worm nests, and others on worm nests ; no positive result was obtained. Ingestion of embryos by a calf also gave negative results, as did a subcutaneous injection of an emulsion of worm nests into a calf. Lice collected from infected calves and put on a young calf failed to transmit the infection. As regards his experiment with *Stomoxys* he commented that the embryos in the nests might be immature and that was probably why they did not develop in that fly. Besides *Stomoxys*, he also suggested *Culicelsa vigilax* as being a probable transmitter, as he found embryos of *O. gibsoni* in a thickened area of the skin of a calf, and their depth below the surface was in many instances much less than the length of the proboscis of *S. calcitrans*. Mallophaga picked from the skin of infected cattle and dissected showed no embryos.

McEachran and Hill (1914) experimented during the period from January to July and found that calves placed in screened and open

pens about 30 yards away from infected cattle did not become infected, whilst calves grazing with infected dairy herds became infected within six months. They stated that the housing arrangement of the calves and the fact that they did not become infected, indicated that the vector was not a biting or flying insect or even an ordinary skin parasite which travels a short distance. The vector seemed to be in the ground and part of the life history of the parasite may be passed in or on the ground. Two calves in the open pen were observed to be attacked by two species of Tabanidae, *Stomoxys*, *Lyperosia* and *Haematopinus tuberculatus*. They fed a tabanid fly on a nodule and dissection later gave negative results.

Dickenson and Hill (1917) carried out a similar series of experiments to those mentioned above of McEachran and Hill, but during the period from July to February ; they got exactly similar results. The calves in the open pen were frequently bitten by two species of Tabanidae, *Stomoxys calcitrans* and *Lyperosia exigua*. It was certain that they were also bitten by several species of *Culicoides*, and probably sandflies ; they were infested with ticks. The authors excluded the species of flies and ticks mentioned above, and also any aquatic forms, as the drinking-water for the screened calves was obtained from a bored well 116 feet deep. They commented, however, that certain species of tabanids rarely attack stock under a roof.

Cleland, Dodd and Ferguson (1917) allowed a large number of *Stomoxys calcitrans* to feed on infected cattle or worm nests and then allowed them to feed on two calves. Subsequent *post mortem* examination failed to show any infection. A similar experiment with negative results was carried out with over 2,000 mosquitos nearly all belonging to the species *Culicelsa vigilax*. Another calf was allowed to be bitten by a few tabanids caught on infected animals with subsequent negative findings. A control calf which was exposed naturally to the attacks of flies, and indirect contact with infected cattle, was subsequently found infected.

Embryos were found unable to survive for 24 hours in cowdung. They concluded by deciding against *Stomoxys* and mosquitos as insect vectors and said that it was probably some tabanid, as the distribution of Onchocerciasis seemed to correspond with the prevalence of tabanids.

Johnston and Bancroft (1920) experimented for the transmission

with some species of tabanidae, *Musca* and *Fannia* with negative results.

Cilento (1923) reported that a tabanid fly was observed to alight on a cow and attach itself for about five minutes; on examination of the site bitten there were no nodules, but a month later a nodule as large as a rice grain was noticed at that site. No further information was given about this nodule.

In a pouch in the skin of a bullock were placed the heads of six tabanid flies in which unidentified nematodes were found in the neighbourhood of the proboscis. A month later two nodules of the size of rice grains were found at the site of the operation, one of which later, attained the size of an almond. This was dissected out and considered to be an onchocerca nodule. Later on, four other nodules were found within 1½ inches of the site of the operation.

On another occasion a tabanid was observed biting a bullock, and on examination at the time no nodules were found. The animal was killed a month later and at the site of the bite of the tabanid fly a coiled worm, 7 inches long, in a condition of commencing encapsulation was found. The worm was identified as *Onchocerca*.

The above data were quoted to Cilento by a meat inspector.

Blacklock (1927) stated that *Simulium* is by no means unrepresented in Australia, several species having been described, and a species in particular is very abundant in the south-eastern parts of South Australia, occurring in thousands and causing serious annoyance to horses and cattle. He suggested that *Simulium* may be the carrier of *O. gibsoni* in Australia.

Cleland (1927) in the form of a reply to the above statement of Blacklock said that he, as one of the principals engaged for some years in the study of *O. gibsoni*, in endeavouring to ascertain its possible means of transmission, '. . . can state authoritatively that transmission occurs in districts entirely free, so far as careful investigations showed, from any species of *Simulium*.'

ENDEMIC CENTRES OF HUMAN ONCHOCERCA

O. volvulus is found endemic in tropical Africa. It produces tumours under the skin, these being mostly situated on the chest, the crests of the ilium and around the great trochanters and the lower limbs. The microfilariae, besides being in the tumours, are

always present in the skin in these situations, especially around the waist ; a fair number of cases show this skin infection without the presence of tumours.

The chief endemic centres are the African Republic of Liberia, Belgian Congo, French Equatorial Africa, French Congo, French Sudan, Senegal, Nigeria, Gold Coast and Sierra Leone.

O. caecutiens is endemic in tropical America in Guatemala and Mexico. It produces tumours mostly on the head and the embryos also infect the skin.

ENDEMIC CENTRES OF ANIMAL ONCHOCERCA

O. gibsoni is prevalent in Australia including Queensland. It produces tumours mostly in the brisket of cattle and the hind limbs ; the skin of infected cattle is found also to be heavily infected with embryos. It is endemic in the Malay Archipelago, including Java and Siam, producing similar lesions ; also in Southern Rhodesia, and it is reported to be spreading from this as a centre to the Union of South Africa. In Rhodesia, however, the infection is mild as the tumours occur only on the brisket of cattle and in small numbers.

O. indica is endemic in India and Ceylon producing nodules in cattle and buffaloes.

O. gutturosa occurs in Queensland, New South Wales and the Northern Territory in cattle. It is very prevalent in Egypt and the Sudan affecting the cervical ligament of cattle. It also affects the cattle in Tunis and Algeria.

O. bovis is found in France in cattle, producing no nodules ; it occurs in the region of the femoro-tibial articulation. It is very prevalent in the Argentine Republic and Uruguay, causing lesions which are mostly situated in the cervical ligament ; about one-fifth of the lesions occur on the external surface of the great trochanter. In Madagascar nearly all the cattle are affected, the lesions being mostly in connective tissue adjacent to the ligaments and tendons, especially ligamentum nuchae.

O. armillata affects nearly 80 per cent. of Egyptian and Sudanese cattle and to a less extent buffaloes, affecting the aorta and causing loss of elasticity by the formation of calcareous plates, wrinkling of the vessel wall and sometimes aneurysm. It is endemic also in

Upper Senegal, Nigeria, India and the Malay States, including Sumatra and Annam, affecting the aorta of cattle and sometimes buffaloes, in all these centres.

O. lienalis occurs in the United States of America in the capsule of the spleen and also in the spleen of cattle in Queensland, New South Wales and the Northern Territory.

O. fasciata affects camels in Western Australia, in India and also in Egypt and the Sudan, causing subcutaneous fibrous growths.

O. reticulata is reported once in Egypt in the aortic wall of an Arab horse which showed a fusiform aneurysm.

DISTRIBUTION OF THE *SIMULIUM* IN THE ENDEMIC CENTRES OF HUMAN AND BOVINE ONCHOCERCA

Australia : Lea (1917) recorded a species of *Simulium* to be very abundant in the south-eastern part of South Australia, occurring in thousands and causing serious annoyance to cattle and horses.

Taylor (1918) recorded the presence of three species of *Simulium*.

Hill (1919) reported its presence in Queensland.

Tonnoir (1925) reported the presence of five species of *Simulium* and sixteen species *Austrosimulium*, seven of which occur in New Zealand ; '*Austrosimulium*,' he said, 'is restricted to the Indo-Australian region.' In Australia, including Tasmania, five species of *Simulium* and nine *Austrosimulium* occur.

Java : Friederichs (1925) reported *Simulium* in Java attacking the ears of cattle, buffaloes, and horses.

Sumatra : Edwards (1925) reported the presence of *Simulium* in Sumatra.

India : Brunetti (1919) reported a number of Simuliids some of which bite viciously. Some species are widely distributed and occur as far south as Ceylon. Others occur in Assam and Java.

In 1917 the same author reported three species in the Simla district.

Egypt : One species is stated by Willcocks (1917) to be recorded from Assuan.

Tunis : Chatton and Blanc (1917) reported *Simulium* in Tunis attacking the gundi.

France : Buysson (1921) reported a species causing discomfort

to cattle and horses while out in the pasture in March by continually attacking the inside of their ears. He suggested that it may convey foot and mouth disease and septic conditions.

Argentina : Lutz (1918) and others reported the presence of several species of *Simulium* in Argentina.

South Africa : Fuller (1913) stated that a number of species of *Simulium* exist throughout South Africa, the flies being local. In some districts they attack the sheep and the ears of horses and there is one species which is a great pest to poultry around Capetown.

Nigeria : Connal and Corkhill (1916) recorded the presence of *S. damnosum.*

Pomeroy (1922) reported the presence of many species.

Sierra Leone : Blacklock (1926) demonstrated the life cycle of *O. volvulus* in *S. damnosum.*

Liberia : The Harvard Expedition demonstrated the life cycle of *O. volvulus* in *S. damnosum* in 1926-27.

French Sudan : and French Equatorial Africa : Laigret (1929) mentioned the presence of *Simulium.*

Congo : Ringenbach (1914) found *Simulium* larvae in almost all the water courses on a journey in Middle Congo and Gaboon.

Ouzilleau (1915) stated that *Simulium* is very common along the Mbomu River.

Cameroons : *S. damnosum* has been reported there.

Guatemala : Calderón reported the presence of *Simulium* which are very prevalent in the endemic *caecutiens* zones.

Mexico : Fülleborn (1923) reported the presence of *Simulium* there and Hoffman (1930) followed the development of *O. caecutiens* embryos in *S. mooseri.*

The reader can see from the historical review of the experimental works on the transmission of both *O. volvulus* and *O. gibsoni* that a good deal of research had been done before 1925, but no positive result established.

By his studies of the bionomics of the fly Blacklock was enabled to explain the preponderance of the tumours and the embryos infected areas of the skin around the waist and hips and lower limbs. Thus he found that *Simulium* does not like bright sunlight, and will not travel in it, even to feed, for more than one or two yards. It prefers the shade either while resting on the grass or while biting,

and this habit of the vector explains the paucity of the lesions on the head or upper limbs in localities where these are always exposed to bright sunlight.

In the case of *O. caecutiens* which was considered a separate species only because of the preponderance of the tumours on the head, he adduced evidence that it is *O. volvulus* and concluded that *Simulium* is its vector; the occurrence of the lesions on the head are due to the difference in the conditions in which the Americans are living in the endemic areas; these conditions include work under overhead shade which gives the *Simulium* an opportunity to bite the body at higher levels. His prophecy has been quite recently proved correct by Hoffman (1930), who reported the development of *O. caecutiens* in *Simulium mooseri*. After the publication of Blacklock's researches, workers on the transmission problems of human onchocerciasis concentrated their efforts on *Simulium*, and already the life cycle has been confirmed in that fly by the Harvard Expedition to Liberia (1930).

The Australian workers on *O. gibsoni* did not take the problem of *Simulium* seriously, and although they appear to have experimented on every possible vector except *Simulium*, their efforts have so far been fruitless. The writer thinks it more than probable that *Simulium* is the insect vector of *O. gibsoni* for the following reasons, viz. :—The pathological manifestations produced by *O. gibsoni* in cattle bear a great similarity to those produced by *O. volvulus* in man. Thus both produce tumours in their host and in both the skin is found to be heavily infected; moreover, the embryos are absent from the blood of infected patients or animals. The sites of the tumours in both diseases are quite comparable, as the majority of the *gibsoni* tumours occur on the brisket and hind limbs, i.e., in shaded portions of the body of the animals, the situations most commonly affected by *O. volvulus*. Thus if *Simulium* is the transmitter, it would attack such regions as the brisket and hind limbs since they are shaded, and the fly need not expose itself to sunlight.

Moreover, *Simulium* is well represented in Australia, as four authors record it in that continent, sometimes in enormous numbers and attacking cattle and horses. Besides, *Simulium*, as Blacklock demonstrated, is not a fly which would declare its presence like other flies and attract attention. They may be present in large numbers

in a plot of grass, and an observer may stand beside this for a long time and not see them. It is worth an attempt on the part of the Australian workers to make an exhaustive survey of the distribution of the different species of *Simulium* in that continent, and to carry out experimental work on the transmission of *O. gibsoni* with them. In this way they may obtain a solution of the problem of transmission, and thus save large sums annually, which are being lost through *O. gibsoni* infestations.

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STUDIES IN CHEMOTHERAPY

VI. — THE PRODUCTION OF RESISTANT STRAINS BY EXPOSURE OF TRYPANOSOMES TO REDUCED TRYPARSAMIDE *IN VITRO*

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In a previous paper (1931) we have shown that a normal strain of trypanosomes will rapidly absorb an organic trivalent arsenical compound, e.g., reduced tryparsamide, when exposed *in vitro* to a solution of the drug in a nutrient medium ; and furthermore that a resistant strain of the same trypanosome is incapable of absorbing any appreciable quantity of the drug when exposed to a similar solution.

This observation led us to enquire whether a single exposure *in vitro* of a normal strain of trypanosomes to a solution of reduced tryparsamide, or repeated exposures—each exposure *in vitro* alternating with the passage of the washed parasites through a normal mouse—would result in the production of a strain resistant to the drug in question.

EXPERIMENT. A mouse heavily infected with *T. rhodesiense* (normal strain) was killed and its blood added to citrated Ringer-glucose solution. The citrated blood was then centrifuged at low speed, and the supernatant fluid containing the trypanosomes withdrawn from the deposit of red cells. After repeating this process to remove the remaining red cells the supernatant fluid was centrifuged at high speed to throw down the trypanosomes, which were then washed with a large volume of nutrient medium, again thrown down in the high speed centrifuge and finally taken up in 0·5 c.c. of nutrient medium (equal parts of rabbit serum, heated at 62° to 64° C. for 30 minutes, and Ringer-glucose solution).

An equal volume (0·1 c.c.) of this suspension of trypanosomes was then added to each of five tubes containing 1 c.c. of nutrient medium in which reduced tryparsamide had been dissolved in the proportions shown in Table IA.

The initial number of trypanosomes in each of the five tubes was found

to be about 10,000 per 256 squares of the haemocytometer scale. After incubation at 37° C. for one hour, the trypanosomes were again counted with the results shewn in the Table.

The tubes were then centrifuged at high speed and the supernatant fluid removed; fresh nutrient medium was added to the deposit of trypanosomes and even suspensions made with the object of obtaining thorough washing of the trypanosomes. These procedures were then repeated three times, and finally the trypanosomes, thus washed four times, were suspended in 1 c.c. of nutrient medium. A count of the number of trypanosomes in each tube was made with the results shewn in Table IA.

TABLE IA

Showing the result of exposure *in vitro* of suspensions of *T. rhodesiense* (normal strain) to various concentrations of reduced tryparsamide.

Tube	Concentration of reduced tryparsamide in nutrient medium	Number of living trypanosomes per 256 squares of haemocytometer scale		
		Start	After 1 hour at 37° C.	After washing
1	1 : 12,800,000	10,000	9,000	9,000
2	1 : 3,200,000		9,500	9,000
3	1 : 800,000		3,000	1,000
4	1 : 200,000		2	0
5	Control		9,500	9,000

From this Table it appears that under the conditions of experiment (i.e., period of exposure at 37° C., 1 hour; initial concentration of trypanosomes about 10,000 per 256 squares of the haemocytometer scale) concentrations of 1 : 12,800,000 and 1 : 3,200,000 reduced tryparsamide did not suffice to destroy any appreciable number of trypanosomes. A concentration of 1 : 800,000, however, reduced the number of trypanosomes from about 10,000 to 3,000 during the hour of exposure to the drug, and during the period occupied by washing (30 minutes) the number fell still further to about 1,000. Finally, a concentration of 1 : 200,000 reduced tryparsamide destroyed practically all the trypanosomes within the hour.

In order to ascertain whether the washed trypanosomes in Tubes 1, 2, and 3 (Table IA) had absorbed lethal quantities of the drug which would eventually destroy them, the following observations were made.

Firstly, small quantities of the suspensions of washed trypanosomes in Tubes 1, 2, and 3 were so diluted with fresh nutrient medium that in each instance the concentration of trypanosomes was reduced to approximately 100 per 256 squares of the haemocytometer scale. These relatively weak suspensions were then incubated at 37° C. and examined from time to time with the results shewn in Table IB.

TABLE IB

Showing the longevity of suspensions of washed trypanosomes previously exposed for 1 hour at 37° C. to various concentrations of reduced tryparsamide. (Table IA.)

Tube	Source of washed trypanosomes suspended in fresh nutrient medium	Concentration of reduced tryparsamide to which the trypanosomes had been exposed for 1 hour before washing	Number of trypanosomes per 256 squares of the haemocytometer scale				
			Start	1 hour	8 hours	24 hours	48 hours
A	Tube 1 (Table IA)	1 : 12,800,000	100	...	102	110	54
B	,, 2 ,,	1 : 3,200,000	110	...	20	0	...
C	,, 3 ,,	1 : 800,000	*100	17	0	...	...
D	,, 5 ,,	Control	100	...	96	94	56

* Many degenerating forms seen.

Secondly, groups of mice were inoculated with the remainder of the trypanosome suspensions in Tubes 1, 2, 3, and 4 (Table IA) with the results set forth in Table IC.

From these experiments the following inferences can be drawn :—

(1) A concentration of 1 : 200,000 reduced tryparsamide sufficed under the conditions of the experiment to destroy all the parasites, because, firstly, practically no living forms were found in the deposit after centrifuging, and secondly, inoculation of the washed deposit into four mice (No. 986) failed to produce infection in any of the animals.

(2) A concentration of 1 : 800,000 reduced tryparsamide failed to destroy all the parasites within 1 hour at 37° C., but greatly reduced their number. Apparently, most of those not destroyed within this period had absorbed a lethal quantity of the drug, because,

even after washing, the number of living trypanosomes had fallen still further, and after an hour's incubation of the washed parasites in fresh nutrient medium only comparatively few were found to be alive ; after 8 hours incubation no living forms were found.

On the other hand, it is quite clear that 1 : 800,000 reduced tryparsamide for 1 hour at 37° C. failed to kill all the parasites,

TABLE Ic

Showing the result of the injection of mice with the suspensions of washed trypanosomes previously exposed to various concentrations of reduced tryparsamide (Table Ia) ; and the effect of treatment of the infected mice.

Mouse	Inoculum	Results of examination of blood of mice, and effect of treatment								
		Day after inoculation								
		2	3	4	5	6	7	9	10	11-30
983 A	0·25 c.c. of a 10-fold dilution of Tube 1 (Table Ia)	15	++ Experiment 2, Series 1	...	...	...	...	...	...	...
B		5	++ R.T. 0·05	—	—	—	10	Dead	...	...
C		+	Dead	...	...	...	...	...	...	...
D		1	++ R.T. 0·075	—	—	—	3	Dead	...	...
984 A	0·25 c.c. of a 10-fold dilution of Tube 2 (Table Ia)	3	++ R.T. 0·075	—	—	—	—	++	Dead	...
B		3	++ R.T. 0·1	—	—	—	1	++	Dead	...
C		15	+++	Dead	...	...	...	...	...	...
D		1/40	5 R.T. 0·05	—	—	—	+	Dead	...	...
985 A	0·25 c.c. of undiluted Tube 3 (Table Ia)	—	1/10	5	++ R.T. 0·05	—	1/10	++	Dead	...
B		—	1/8	8	Dead	...	...	...	...	...
C		—	1/10	10	+++ Experiment 2, Series 2	...	...	...	...	...
D		—	1/3	20 R.T. 0·05	—	—	—	++	Dead	...
986 A	0·25 c.c. of undiluted Tube 4 (Table Ia)	—	—	—	—	—	—	—	—	—
B		—	—	—	—	—	—	—	—	—
C		—	—	—	—	—	—	—	—	—
D		—	—	—	—	—	—	—	—	—

N.B.—1, 15, +, etc. = number of trypanosomes per microscope field.
— = blood negative.
R.T. 0·05 = reduced tryparsamide 0·05 mgm. per 20 gm. of mouse.

because inoculation of the washed deposit into four mice (No. 985) produced infection in every case, although, considering the number of parasites injected, the incubation period was somewhat prolonged.

(3) For a similar reason it is clear that when the concentration of reduced tryparsamide was 1 : 3,200,000 a large number of trypanosomes had absorbed within an hour a lethal quantity of the drug.

(4) So far as one could ascertain, however, a concentration of 1 : 12,800,000 reduced tryparsamide exerted no harmful effect on the suspension. The washed trypanosomes subsequently incubated in fresh nutrient medium survived as well as the controls; and the four mice (No. 983) inoculated with small portions of the suspension of washed trypanosomes all became infected after the customary short incubation period.

It thus becomes evident that under the foregoing conditions of experiment (period of action of drug at 37° C., 1 hour; concentration of trypanosomes, 10,000 per 256 squares of the haemocytometer scale), 1 : 12,800,000 reduced tryparsamide is the highest concentration which fails to exert any discernible harmful action on the trypanosomes; and 1 : 800,000 is the highest concentration which fails to destroy completely the entire number of trypanosomes.

Reference to Table Ic shows that the infections in mice, produced by the washed trypanosomes exposed to 1 : 12,800,000, 1 : 3,200,000 and 1 : 800,000 reduced tryparsamide, respectively, failed to exhibit any signs of drug resistance, since the peripheral blood of all the mice was sterilized by such minute doses of reduced tryparsamide as 0·05 mgm. and 0·075 mgm. per 20 gm. of mouse, which are but slightly greater than the minimum effective dose (M.E.D.) for the normal strain. It seems fairly clear, therefore, that a single exposure of trypanosomes *in vitro* to any concentration of reduced tryparsamide does not suffice to enhance appreciably the resistance of the trypanosomes to the drug.

Having reached this position we decided to ascertain whether a series of exposures of trypanosomes *in vitro* to reduced tryparsamide, alternating with passage of the parasites through normal mice, would eventually result in the production of a tryparsamide-resistant strain.

With this object in view, two parallel series of experiments were conducted. In the first, the concentration of reduced tryparsamide

was always 1 : 12,800,000, i.e., the highest concentration which failed to exert any harmful effect on the suspension of normal trypanosomes. In the second, the concentration of reduced tryparsamide was initially 1 : 800,000, but as the parasites developed resistance, this was correspondingly increased, so that the concentration of the drug was always maintained at the highest level which just failed to destroy all the parasites in the suspension.

For the sake of simplicity we shall at first confine ourselves to a consideration of the second series of experiments in which the relatively concentrated solutions of reduced tryparsamide were employed. These experiments commenced with the trypanosomes from Mouse 985C (Table Ic). The results of the individual experiments comprising this series are summarized in Tables II to XIII.

The general technique employed in each experiment was the same as that already described. A heavy suspension of trypanosomes obtained from one of the mice from a previous experiment was exposed *in vitro* for 1 hour at 37° C. to a solution of reduced tryparsamide in nutrient medium. The trypanosomes were then deposited by means of high speed centrifugalization, and after being washed four times in nutrient medium were injected into a group of four mice. When these mice became infected, the trypanosomes from one of them were used for the next experiment, and the degree of resistance of the infections in the other mice was tested by the administration of various doses of reduced tryparsamide.

It will be noticed that in the first four experiments of this series the concentration of reduced tryparsamide employed was 1 : 800,000 and 1 : 200,000. As, however, the latter concentration invariably destroyed all the trypanosomes of the suspension within an hour at 37° C., the strain in each case was carried on from the suspension exposed to 1 : 800,000 reduced tryparsamide. In Experiment 5, for the first time, the suspension subjected to a solution of 1 : 200,000 reduced tryparsamide was not entirely destroyed, and the mice injected with this suspension became infected. The same thing is found in Experiment 6, the trypanosomes for which were obtained from a mouse of the previous experiment which had been injected with the trypanosomes exposed *in vitro* to a concentration of 1 : 200,000 reduced tryparsamide. In Experiment 5 there is thus, for the first time, definite evidence of increased resistance on the part

of the trypanosomes. In Experiment 6 the degree of resistance is very pronounced. In Experiments 7, 8, and 9 it was found that the trypanosome suspensions became progressively capable of withstanding ever greater concentrations of the drug, until, in Experiment 9, a solution of the drug of relatively such enormous concentration as 1 : 6,250 failed to destroy all the parasites in the suspension, and three of four mice inoculated with the treated suspension became infected. Although the experiment was carried beyond this point (*vide* Tables X to XIII), no evidence was obtained of any further development of resistance, and the series was consequently closed.

TABLE IIA

Showing the result of Exposure II.

Tube	Concentration of reduced tryparsamide in nutrient medium	Source of trypanosomes	Number of living trypanosomes per 256 squares of the haemocytometer scale		
			Start	After 1 hour at 37° C.	After washing
1	1 : 800,000	Mouse 985 C.	5,500	4	3
2	1 : 200,000	(Table Ic)	5,500	0	...

TABLE IIB

Showing the result of injection of mice with washed trypanosomes after Exposure II, and the effect of treatment of the infected mice.

Mouse	Inoculum	Results of examination of blood of mice, and effect of treatment											
		Day after inoculation											
		3	4	5	6	7	8	9	11	12	13	14	15-40
998 A	0·25 c.c. of undiluted Tube 1 (Table IIA)	—	—	—	—	1/20	3 R.T. 0·075	—	—	—	—	—	—
B		—	—	1/5	5	++ Experiment 3	...	...	...	...	...	...	...
C		—	—	1/20	6	+ R.T. 0·05	—	1/20	20	+	++	Dead	...
D		—	—	1/8	10	++ R.T. 0·1	—	—	Dead	...	...	...	...

TABLE IIIA

Showing the result of Exposure III.

Tube	Concentration of reduced tryparsamide in nutrient medium	Source of trypanosomes	Number of living trypanosomes per 256 squares of the haemocytometer scale		
			Start	After 1 hour at 37° C.	After washing
1	1 : 800,000	Mouse 998 B	12,000	100	100
2	1 : 200,000	(Table IIB)	12,000	0	...

TABLE IIIB.

Showing the result of injection of mice with washed trypanosomes after Exposure III; and the effect of treatment of the infected mice.

Mouse	Inoculum	Results of examination of blood of mice, and effect of treatment										
		Day after inoculation										
		2	3	4	5	6	7	8	9	11	12	13
20 A	0·125 c.c. of undiluted Tube I (Table IIIA)	—	—	1/5	20	+++ Experiment 4	...	...	...	...	...	...
B		—	—	1/10	30 R.T. 0·05	—	—	1/2	20	Dead	...	...
C		—	—	1/2	+ R.T. 0·05	—	—	—	—	10	+++	Dead
D		—	—	1	+ R.T. 0·075	—	—	—	—	5	+++	Dead

TABLE IVA

Showing the result of Exposure IV

Tube	Concentration of reduced tryparsamide in nutrient medium	Source of trypanosomes	Number of living trypanosomes per 256 squares of the haemocytometer scale		
			Start	After 1 hour at 37° C.	After washing
1	1 : 800,000	Mouse 20A	15,000	300	...
2	1 : 200,000	(Table IIIB)	15,000	0	...

TABLE IVB

Showing the result of injection of mice with washed trypanosomes after Exposure IV ; and the effect of treatment of the infected mice.

Mouse	Inoculum	Results of examination of blood of mice, and effect of treatment.											
		Day after inoculation											
		3	4	5	6	7	8	9	10	12	13	14	15
42 A	0·25 c.c. of undiluted Tube 1 (Table IVA)	—	—	1/30	20	+++ Experiment 5	...	...	...	...	...	...	...
B		—	—	1/5	+ R.T. 0·05	—	—	—	1/30	+	+++	Dead	...
C		—	—	1	30	+++	Dead	...	...	...	...	...	...
D		—	—	5	+ R.T. 0·075	—	—	—	—	1/3	+	+++	Dead

TABLE Va

Showing result of Exposure V.

Tube	Concentration of reduced tryparsamide in nutrient medium	Source of trypanosomes	Number of living trypanosomes per 256 squares of the haemocytometer scale		
			Start	After 1 hour at 37° C.	After washing
1	1 : 800,000	Mouse 42 A	12,000	3,400	3,000
2	1 : 200,000	(Table IVB)	12,000	1,000	1,000

TABLE Vb

Showing the result of injection of mice with washed trypanosomes after Exposure V; and the effect of treatment of the infected mice.

Mouse	Inoculum	Results of examination of blood of mice, and effect of treatment				
		Day after inoculation				
		1	2	3	4	5
64 A	0·25 c.c. of a 10-fold dilution of Tube 1 (Table Va)	—	1/15	20 R.T. 0·05	+++	Dead
B		—	5	+ R.T. 0·075	Dead	...
C		—	5	++ R.T. 0·075	Dead	...
D		—	5	++ R.T. 0·125	Dead	...
65 A	0·25 c.c. of undiluted Tube 2 (Table Va)	1/50	5	++ R.T. 0·075	Dead	...
B		1	+ R.T. 0·05	+++	Dead	...
C		1	15	+++ Experiment 6	...	...
D		1/30	2	++ R.T. 0·125	Dead	...

TABLE VIA

Showing the result of Exposure VI.

Tube	Concentration of reduced tryparsamide in nutrient medium	Source of trypanosomes	Number of living trypanosomes per 256 squares of the haemocytometer scale		
			Start	After 1 hour at 37° C.	After washing
1	1 : 800,000	Mouse 65C	7,400	7,000	7,000
2	1 : 200,000	(Table VB)	7,400	2,500	2,000

TABLE VIB

Showing the result of injection of mice with washed trypanosomes after Exposure VI; and the effect of treatment of the infected mice

Mouse	Inoculum	Results of examination of blood of mice, and effect of treatment								
		Day after inoculation								
		2	3	4	5	6	7	8	9	11
91 A	0·25 c.c. of a 10-fold dilution of Tube 1 (Table VIA)	1/50	1/20	1 R.T. 0·25	++	Dead	...	...	...	...
B		1/5	1	++ R.T. 1·0	1/2	10	1/5	+	Dead	...
C		1/10	2	+ R.T. 0·5	20	++	Dead	...	...	...
D		1/10 R.T. 0·125	2	+ R.T. 2·0	1/10	−	−	−	+	Dead
92 A	0·25 c.c. of a 3-fold dilution of Tube 2 (Table VIA)	−	−	−	1/2	+ R.T. 1·0	20	+++	Dead	...
B		−	−	−	1	++	Dead	...	...	...
C		−	−	−	1	++	Dead	...	...	...
D		−	−	1/20	20	+++ Experiment 7	...	...	...	...

TABLE VIIA

Showing the result of Exposure VII.

Tube	Concentration of reduced tryparsamide in nutrient medium	Source of trypanosomes	Number of living trypanosomes per 256 squares on the haemocytometer scale		
			Start	After 1 hour at 37° C	After washing
1	1 : 200,000	Mouse 92D.	1,000	850	...
2	1 : 100,000	(Table VIB)	1,000	66	...
3	1 : 50,000		1,000	8	...

TABLE VIIB

Showing the result of injection of mice with washed trypanosomes after Exposure VII; and the effect of treatment of the infected mice.

Mouse	Inoculum	Results of examination of blood, and effect of treatment						
		Day after inoculation						
		2	4	5	6	7	9	10
111 A	0·25 c.c. of a 10-fold dilution of Tube 1 (Table VIIA)	−	+ R.T. 1·25	Dead	...	...	...	...
B		−	5 R.T. 2·0	++	Dead	...	...	...
C		−	+ R.T. 2·5	Dead	...	...	...	...
D		−	10	+ R.T. 2·5	Dead	...	...	...
112 A	0·25 c.c. of a 2-fold dilution of Tube 2 (Table VIIA)	−	++ R.T. 2·5	++	Dead	...	...	...
B		−	++ R.T. 2·0	+++	Dead	...	...	...
C		−	20 R.T. 1·25	+++	Dead	...	...	...
D		−	1/5	+ R.T. 2·5	+++	Dead	...	...
113 A	0·25 c.c. of a 2-fold dilution of Tube 3 (Table VIIA)	−	+ R.T. 2·0	Dead	...	...	...	...
B		−	20	+++ Experiment 8	...	...	...	...
C		−	10	++ R.T. 2·5	+++	Dead	...	...
D		−	−	1/20 R.T. 2·5	1	1	+++	Dead

TABLE VIIIA

Showing the result of Exposure VIII.

Tube	Concentration of reduced tryparsamide in nutrient medium	Source of trypanosomes	Number of living trypanosomes per 256 squares of the haemocytometer scale			Survival*		
			Start	After 1 hour at 37° C.	After washing	Start	4 hours	24 hours
1	1 : 100,000	Mouse 113 B.	5,600	6,000	6,000	120	110	88
2	1 : 50,000	(Table VIIB)	5,600	5,000	5,500	120	110	86
3	1 : 25,000		5,600	4,200	4,000	120	72	1

* This shows the longevity of dilute suspensions of the washed trypanosomes in nutrient medium (*vice* Table IB).

TABLE VIIIB

Showing the results of injection of mice with washed trypanosomes after Exposure VIII; and the effect of treatment of the infected mice.

Mouse	Inoculum	Results of examination of blood, and effect of treatment — Day after inoculation										
		1	2	3	4	5	6	7	8	9	10	11-30
132 A	0·25 c.c. of a 10-fold dilution of Tube 1 (Table VIIIA)	5	10 N.A.B. 4·0	Dead	...	...	...	...	...	...	...	...
B		—	1/5 R.T. 2·5	+	Dead	...	...	...	...	...	...	...
C		1/10	15 AP.G. 0·5	++	Dead	...	...	...	...	...	...	...
D		1/5	10 A.P.G. 1.0	—	—	—	—	—	—	—	—	—
133 A	0·25 c.c of a 10-fold dilution of Tube 2 (Table VIIIA)	—	—	1/3	+ A.P.G. 0·5	—	20	++	Dead	...	...	...
B		1/10	1 R.T. 2·5	20	Dead	...	...	...	...	...	...	...
C		—	—	1	+ A.P.G. 0·75	+++	Dead	...	...	...	...	...
D		—	1/5 R.T. 2·0	10	++	Dead	...	...	...	...	...	...
134 A	0·25 c.c. of a 10-fold dilution of Tube 3 (Table VIIIA)	—	—	1/5	10 A.P.G. 0·5	—	1/5	1/2	15	Dead	...	...
B		—	—	10	+ A.P.G. 0·75	—	—	1/5	10	+++	Dead	...
C		—	1/10	20	+++ Experiment 9	...	...	...	...	...	...	...
D		—	1/10	5	++	Dead	...	...	...	...	...	...

N.A.B. 4·0 = Novarsenobillon 4·0 mgm. per 20 gm. of mouse.
A.P.G. 0·5 = Arsenophenylglycine 0·5 mgm. per 20 gm. of mouse.

TABLE IXA

Showing the result of Exposure IX.

Tube	Concentration of reduced tryparsamide in nutrient medium	Source of trypanosomes	Number of living trypanosomes per 256 squares of the haemocytometer scale		
			Start	After 1 hour at 37° C.	After washing
1	1 : 12,500	Mouse 134 C.	3,300	2,700	...
2	1 : 6,250	Table VIIIB)	3,300	2,500	...

TABLE IXB

Showing the result of injection of mice with washed trypanosomes after Exposure IX; and the effect of treatment of the infected mice.

Mouse	Inoculum	Results of examination of blood, and effect of treatment											
		Day after inoculation											
		2	3	4	5	6	7	9	10	11	12	13	14-30
152 A	0·25 c.c. of a 10-fold dilution of Tube 1 (Table IXA)	—	—	1	5	20 A.P.G. 0·5	8	+	+++	Dead	...	...	...
B		—	—	2	15	++ A.P.G. 0·75	1/5	++	Dead	...	...	...	...
C		—	1	10	++	Dead	...	...	...	...	...	...	...
D		—	—	1/20	1	+ A.P.G. 0·75	—	1	++	+++	+++	Dead	...
153 A	0·25 c.c. of a 5-fold dilution of Tube 2 (Table IXA)	—	—	—	—	—	2 A.P.G. 0·75	+	+++	Dead	...	...	...
B		—	—	—	—	—	—	—	—	—	—	—	—
C		—	—	—	—	5	+ Experiment 10	...	...	...	...	...	...
D		—	—	—	—	1/10	3 A.P.G. 0·75	5	++	+++	Dead	...	...

TABLE XA

Showing the result of Exposure X.

Tube	Concentration of reduced tryparsamide in nutrient medium	Source of trypanosomes	Number of living trypanosomes per 256 squares of the haemocytometer scale		
			Start	After 1 hour at 37° C.	After washing
1	1 : 6,250	Mouse 153 C.	8,000	5,000	...
2	1 : 3,125	(Table IXB)	8,000	6,000	...

TABLE XB

Showing the result of injection of mice with washed trypanosomes after Exposure X ; and the effect of treatment of the infected mice.

Mouse	Inoculum	Results of examination of blood, and effect of treatment												
		Day after inoculation												
		4	5	6	7	9	10	11	12–16	17	18	19	20	21	22–30
169 A	0·125 c.c. of undiluted Tube 1 (Table XA)	−	−	−	−	−	1/20	5 A.P.G. 0·75	−	−	1/30	3	+	Dead	...
B		−	−	−	1	+++ Experiment 11	...	...	...	...	...	...	...	...	...
C		−	−	−	1/20	−	1/10	20 A.P.G. 0·75	−	+	Dead	...	...	...	...
D		−	−	−	−	−	−	−	−	−	−	−	−	−	−
170 A	0·125 c.c. of undiluted Tube 2 (Table XA)	−	−	−	−	−	−	−	−	−	−	−	−	−	−
B		−	−	−	−	−	−	−	−	−	−	−	−	−	−
C		−	−	−	−	−	−	−	−	−	−	−	−	−	−
D		−	−	−	−	−	−	−	−	−	−	−	−	−	−

TABLE XIA

Showing the result of Exposure XI.

Tube	Concentration of reduced tryparsamide in nutrient medium	Source of trypanosomes	Number of living trypanosomes per 256 squares of the haemocytometer scale		
			Start	After 1 hour at 37° C.	After washing
1	1 : 6,250	Mouse 169 B.	1,800	400	400
2	1 : 3,125	(Table XB)	1,800	200	25

TABLE XIB

Showing the result of injection of mice with washed trypanosomes after Exposure XI ; and the effect of treatment of the infected mice.

Mouse	Inoculum	Results of examination of blood, and effect of treatment				
		Day after inoculation				
		4	5	7	8	9-30
207 A	0·25 c.c. of undiluted Tube 1 (Table XIA)	—	—	—	—	—
B		—	—	—	—	—
C		—	—	5	+++ Experiment 12	...
D		—	—	—	—	—
208 A	0·25 c.c. of undiluted Tube 2 (Table XIA)	—	—	—	—	—
B		—	—	—	—	—
C		—	—	—	—	—
D		—	—	—	—	—

TABLE XIIA

Showing the result of Exposure XII.

Tube	Concentration of reduced tryparsamide in nutrient medium	Source of trypanosomes	Number of living trypanosomes per 256 squares of the haemocytometer scale		
			Start	After 1 hour at 37° C.	After washing
1	1 : 6,250	Mouse 207 C.	4,000	4,000	4,000
2	1 : 3,125	(Table XIB)	4,000	500	500

TABLE XIIB

Showing the result of injection of mice with washed trypanosomes after Exposure XII; and the effect of treatment of the infected mice.

Mouse	Inoculum	Results of examination of blood, and effect of treatment									
		Day after inoculation									
		3	4	5	7	8	9	10	11	13	14–30
249 A	0·25 c.c. of undiluted Tube 1 (Table XIIA)	—	1/3	1	20 Emetic 0·75	Dead	...	...	...	...	...
B		—	1/10	+ Experiment 13	...	...	...	...	...	...	...
C		—	—	—	—	1	+ A.P.G. 0·25	10	Dead	...	...
D		—	—	20	+++ Killed for *in vitro* test	...	...	...	...	...	...
250 A	0·25 c.c. of undiluted Tube 2 (Table XIIA)	—	—	—	—	—	—	—	—	Dead	...
B		—	—	—	—	—	—	—	—	—	—
C		—	—	—	—	—	—	—	—	Dead	...
D		—	—	—	—	—	Dead	...	...	...	...

TABLE XIIIA

Showing the result of Exposure XIII.

Tube	Concentration of reduced tryparsamide in nutrient medium	Source of trypanosomes	Number of living trypanosomes per 256 squares of the haemocytometer scale		
			Start	After 1 hour at 37° C.	After washing
1	1 : 6,250	Mouse 249 B.	2,000	1,000	1,000
2	1 : 3,125	(Table XIIB)	2,400	100	100

TABLE XIIIB

Showing the result of injection of mice with washed trypanosomes after Exposure XIII ; and the effect of treatment of the infected mice.

Mouse	Inoculum	Results of examination of blood, and effect of treatment											
		Day after inoculation											
		4	5	7	8	9	10	11	12-14	15	16	17	18-30
294 A	0·25 c.c. of undiluted Tube 1 (Table XIIIA)	—	—	—	—	—	—	—	—	—	—	—	—
B		—	1/10	+++	Dead	...	...	...	...	...	...	...	...
C		—	—	—	—	—	10 Stibenyl 15	10	—	1/20	2	Dead	...
D		—	—	+ Stibenyl 15	Dead	...	...	...	...	...	...	...	...
295 A	0·25 c.c. of undiluted Tube 2 (Table XIIIA)	—	—	—	—	—	—	—	—	—	—	—	—
B		—	—	—	—	—	—	—	—	—	—	—	—
C		—	—	—	—	—	—	—	—	—	—	—	—
D		—	—	—	—	—	—	—	—	—	—	—	—

If we analyse the data presented in this series of Tables, certain facts emerge.

1. After the fourth exposure of the strain to 1 : 800,000 reduced tryparsamide for 1 hour at 37° C., there was no evidence of enhanced resistance of the parasites to the drug : the mice infected with the washed parasites after the fourth exposure to the drug were made negative by such small doses of reduced tryparsamide as 0·05 and 0·075 mgm. per 20 gm. of mouse, i.e., by doses but slightly greater than the M.E.D. for the normal strain.

2. At the time of the fifth exposure of the strain to the drug there was, however, definite evidence of resistance. In the first place, 1 : 200,000 reduced tryparsamide no longer destroyed all the trypanosomes in the suspension, and mice inoculated with the washed trypanosomes became infected ; and in the second place doses varying from 0·05 mgm. to 0·125 mgm. no longer sufficed to make the blood of the infected mice negative.

3. From this point onwards, the resistance of the strain to reduced tryparsamide increased rapidly, so that we were able, in successive experiments, to subject the trypanosome suspensions to ever increasing concentrations of reduced tryparsamide without completely destroying them ; and mice inoculated with the washed suspensions of trypanosomes so treated invariably became infected. Thus, in Experiments 5 and 6 solutions of 1 : 200,000 reduced tryparsamide were used ; in Experiment 7 solutions of 1 : 100,000 and 1 : 50,000 failed to destroy all the trypanosomes. At this point the resistance of the trypanosomes in the infected mice was evidently enormous, since maximum doses of 2·5 mgm. of reduced tryparsamide (i.e., about 75 times the M.E.D.) had no effect on the infection.

4. In Experiment 8, 1 : 25,000 reduced tryparsamide had comparatively little effect on the suspension. In Experiment 10, concentrations of 1 : 6,250 and 1 : 3,125 reduced tryparsamide were employed ; neither of these destroyed all the parasites within an hour ; but whereas the washed trypanosomes, which had been exposed to the former infected three of the four mice into which they were injected, those which had been exposed to the latter failed to infect any of the mice.

5. At this point, the resistance appears to have reached its limit or thereabouts, since three further exposures to 1 : 6,250

reduced tryparsamide failed to produce any appreciable increase in the resistance of the strain.

Subsequent tests made with this resistant strain showed that *in vitro* it proved to be at least 500 times as resistant to reduced tryparsamide as was the normal strain from which it originated, and that infections in mice proved completely resistant to maximum doses of reduced tryparsamide (2·5 mgm. per 20 gm. mouse) and halarsol (0·75 mgm. per 20 gm. mouse); there was likewise pronounced resistance to stibenyl, but, on the contrary, the strain was only slightly resistant to arsenophenylglycine and not at all to Bayer 205. In other words, the strain which was developed as the result of successive short exposures *in vitro* to reduced tryparsamide was indistinguishable from that produced by the conventional method of subjecting a series of infected animals to repeated subcurative doses of the drug.

Let us now return to the first series of experiments in which the concentration of reduced tryparsamide, to which the trypanosome suspensions were exposed, was invariably 1 : 12,800,000. The procedure adopted here was in all respects similar to that in the series just described, except that the strength of the reduced tryparsamide solution remained constant at 1 : 12,800,000; consequently it appears unnecessary to reproduce the protocols in detail, the more so as, owing to the fact that the development of resistance was exceedingly slow, the number of experiments in the series was no less than thirty-six.

At the beginning of the series of experiments the blood of infected mice was cleared by a dose of 0·05 mgm. of reduced tryparsamide per 20 gm. of body weight. After the sixth or seventh exposure such a dose failed at times to clear the blood of the infected mice, and after the eleventh exposure the strain was definitely unaffected by this dose.

After the nineteenth exposure there was evidence of some resistance to 0·125 mgm. per 20 gm. of body weight; and after the twenty-fifth and twenty-sixth exposures the strain was completely resistant to this dose. The resistance gradually increased after successive exposures, until after the thirty-sixth exposure, the resistance of the strain, as judged by experiments on infected mice, was no less than twenty times that of the original strain, i.e., doses of 0·5 mgm. of reduced tryparsamide per 20 gm. of mouse failed to

produce peripheral sterilization. At this point the experiment was stopped.

From these two experiments we draw the following conclusions :—

(1) A highly resistant strain of trypanosomes can be produced by a series of short exposures of the parasites *in vitro* to solutions of reduced tryparsamide, alternating with passage of the drug-laden parasites through mice.

(2) The speed with which resistance develops and the degree of its development depend on the concentration of the solution of reduced tryparsamide. When the concentration of reduced tryparsamide was the highest which could be employed without destroying any appreciable number of the trypanosomes, (i.e., 1 : 12,800,000 in the conditions of the experiment, viz., time of exposure 1 hour at 37° C., number of trypanosomes 10,000 per 256 squares of the haemocytometer scale) no definite increase in resistance was observed until the eleventh exposure, and even after the thirty-sixth exposure the resistance was increased only about twenty times. When, however, the concentration of reduced tryparsamide was the highest which could be employed without killing all the trypanosomes, i.e., 1 : 800,000 in the first four exposures and ever increasing concentrations in subsequent exposures, a considerable degree of resistance was observed after the fifth exposure, and, so far as could be judged from *in vivo* tests, complete resistance after the seventh exposure. Finally, a strain was produced which withstood a concentration of 1 : 6,250 reduced tryparsamide, and which more delicate tests shewed to be no less than 500 times as resistant as the original strain.

These observations have a definite bearing on the question whether the development of drug resistance is primarily due to a process of weeding out the sensitive individuals of a strain of trypanosomes leaving only those naturally resistant which tend to reproduce their like (i.e., selection) ; or to one of gradual change in all or certain of the individuals resulting from the stimulus of frequent exposures of the trypanosomes to suitable concentrations of the drug, thus giving rise to the acquisition of a new character which is transmitted apparently unaltered through subsequent generations (i.e., mutation).

In the course of very numerous observations on the action *in vitro* of various arsenical and antimonial compounds on suspensions

of trypanosomes, we have frequently been impressed with the fact that occasional individual trypanosomes are to be seen apparently uninjured, and in a state of normal activity, some time after the remainder of the parasites have completely disintegrated. Such an observation can only mean that all individual trypanosomes are not equally sensitive to the action of the drug in question and suggests that a process of selection plays a not unimportant part in the initial stages at least of the development of drug resistance. The more sensitive individuals are destroyed by the drug, whilst the more insensitive survive and tend to reproduce a strain less sensitive than the original strain. Such a hypothesis would at first sight appear to afford an adequate explanation why Strain II, which was subjected to relatively high (sublethal) and ever increasing concentrations of reduced tryparsamide, developed resistant characters much more rapidly, and to a much greater degree, than did Strain I, which was exposed to a relatively low (non-lethal) concentration of the drug.

Closer examination of all the facts, however, throws considerable doubt on the adequacy of this hypothesis to afford a complete explanation of the development of drug resistance. The main difficulty in the way of its unqualified acceptance is the behaviour of Strain I. In this case the concentration of reduced tryparsamide invariably employed in the successive exposures of the trypanosome suspension was 1 : 12,800,000, and for reasons already given (Table IA and IB) it is clear that such a concentration of drug failed to cause serious damage to any appreciable number of the individuals in the suspension of normal trypanosomes exposed to it.

Furthermore, even though it be admitted, for the sake of argument, that the concentration of 1 : 12,800,000 reduced tryparsamide may have destroyed some small proportion of the total trypanosomes in the suspensions of the first few experiments of Series I, it is inconceivable that this could have happened in the later experiments when the strain had become several times as resistant as it was at the commencement. Yet, throughout the course of the experiments, the fact clearly stands out that as the result of successive exposures to a concentration of the drug which was probably harmless at the very beginning, and certainly so later on, the strain developed a gradual but steadily increasing degree of resistance until, when the

series of experiments was closed after the thirty-sixth exposure, the resistance was about twenty times what it was at the beginning.

Since then it seems impossible to explain the development of resistance in Strain I on the basis of a process of selection, we have reason to doubt whether this process was really the essential factor responsible for the remarkable degree of resistance developed by Strain II.

The hypothesis, which, in our view, appears to afford the most adequate explanation of all the facts, is that the development of drug resistance by a strain of trypanosomes is fundamentally due to a process of mutation, and that the rapidity and degree of this mutation are dependent primarily upon the concentration of drug to which the trypanosomes are exposed. If the initial concentration of reduced tryparsamide be too great, e.g., 1 : 200,000, all the trypanosomes in the suspension are killed and consequently there is, of course, no development of resistance ; if the concentration be relatively low, e.g., 1 : 12,800,000, the stimulus is weak, and consequently the trypanosomes mutate but slowly ; whilst, if the concentration of the drug be relatively high—but insufficiently so to destroy all the parasites—the stimulus is optimum and the process of mutation is rapid.

In the experiments of Series II, in which relatively high concentrations of reduced tryparsamide were used, the possibility cannot be excluded that the process of mutation may have been aided by one of selection ; and it is indeed conceivable that the individuals, in the original strain, which were least sensitive to the drug were likewise those which most readily underwent mutation. We intend to deal with further observations bearing on this interesting subject in a later paper.

SUMMARY

1. A series of short exposures of a normal strain of *T. rhodesiense* to suitable solutions of reduced tryparsamide in nutrient medium—each exposure to the drug *in vitro* alternating with the passage of the washed parasites, through a normal mouse—sufficed to produce a strain highly resistant to the drug.

2. An enhanced drug resistance was not immediately noticeable,

but first made its appearance only after a number of exposures of the strain to the drug.

3. The speed with which resistance developed, and the degree of its development, depended on the concentration of the solution of reduced tryparsamide to which the parasites were exposed.

4. The optimum concentration of drug for this purpose was found to be the highest employed which failed to destroy all the trypanosomes in the suspension exposed to the drug. By this means a strain of trypanosomes was quickly produced which was at least 500 times as resistant to reduced tryparsamide as was the original strain; it was also found to be exceedingly resistant to halarsol and stibenyl, but only slightly so to arsenophenylglycine, and not at all to Bayer 205. The use of lower concentrations of drug likewise sufficed for the production of a resistant strain of trypanosomes, but the process was much slower and the degree of resistance resulting was much less.

5. The question was considered whether the development of drug resistance by a strain of trypanosomes is the result of a process of *selection*, i.e., the weeding out of the more sensitive individuals, and survival of those naturally resistant which tend always to reproduce their like; or whether it is due to a process of *mutation*, i.e., a gradual change in all, or in certain, individuals resulting from the stimulus of frequent exposures of the strain to suitable concentrations of the drug, thus giving rise to the acquisition of a new character which is transmitted through innumerable subsequent generations.

6. A critical analysis of the facts brought to light in these experiments indicates that the development of drug resistance by a trypanosome is fundamentally the result of a process of mutation, although the possibility cannot be excluded that under certain conditions the process of mutation may be aided by one of selection.

REFERENCE

YORKE, W., MURGATROYD, F., and HAWKING, F. (1931). Studies in Chemotherapy. V.—Preliminary contribution on the nature of drug resistance. *Ann. Trop. Med. & Parasitol.*, **25**, 351.

A NEW SUBSPECIES OF *ANOPHELES FUNESTUS* GILES, FROM SOUTHERN RHODESIA

BY

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The recent study of variation in *Anopheles funestus* made by Leeson (1930), in Southern Rhodesia, showed that, as a whole, the forms of the species occurring there differed in certain important respects from the type form of *funestus* as defined by Christophers and Puri (1931) and as known from Sierra Leone, the type locality. The entire absence of the basal costal interruption and the frequent occurrence of a very extensively pale third vein being the most notable differences.

Through the kindness of Dr. P. A. Buxton and Mr. H. S. Leeson, I have been able to study a considerable number of larvae and adults and some pupae collected by Mr. Leeson, in Southern Rhodesia. Examination of these specimens has revealed the interesting fact that the great majority of the larvae differ from the type form of *funestus* and approach very near *listoni* in the characters recently defined by Christophers and Puri (1931). Moreover, a considerable number of the males examined, especially those with the third vein extensively pale scaled, showed a pale spot at the base of the palpal club, a character found to be absent in typical *funestus* from more Northern localities (Evans, 1930). The pupae also differ considerably from those of the type form of *funestus* and approached that of *listoni* as described by Senevet (1930).

Four of the larvae (out of 72) agreed with those of the type form in essential characters, but all showed certain differences from typical specimens from West and East Africa. It is obvious from Leeson's study of the wing markings of Southern Rhodesian *funestus* that the adults associated with these larvae differ from *funestus* of more Northern regions in the entire absence of the basal costal interruption and, in this character, approach *listoni*. In view of these facts and also of the occurrence among the Rhodesian material of two larvae in which the characters of typical *funestus* are combined

with those of the *listoni*-like form, this latter is described below as a new subspecies of *funestus*. I have consulted Dr. F. W. Edwards about this question and he agrees that this is the best course to adopt.

Owing to Dr. Puri's recent studies of the larvae of *funestus* and its allies, it is possible to compare the present form with them in detail.

ANOPHELES FUNESTUS subsp. *LEESONI*, n.-subsp.

A. funestus Leeson (1930 and 1931), in part.

LARVA. The larva differs from that of *A. funestus* Giles, type form (see Christophers and Puri, 1931, and Evans, 1930) as follows :—

Head pattern without transverse band anteriorly ; when most completely developed the markings are much as illustrated by Evans (1930), fig. 1, G.

Posterior frontal hair (inner sutural) with about four to eight ultimate branches (simple in typical *funestus*).

Abdominal tergal plates variable in depth but usually relatively shallower than in typical *funestus*. The two small posterior plates, when present, not included in the main tergal plates on segments III to VII. Hair ' O ' (of Puri) not arising from tergal plate. In one or two larvae this hair is situated on the edge of the plate in segment VII and in one exceptional case it was well on the plate on V to VII.

The larva differs from that of *A. listoni* (see Puri, 1931) as follows :—

Head pattern in full-grown larvae often very slightly developed, especially anteriorly to the mid frontal hairs, where there may be no dark markings or the two longitudinal stripes may be very faint and much reduced.

Abdomen with pair of small posterior plates sometimes absent or faintly indicated or present on one or two segments only.

Hair ' O ' variable in size and branching, especially on segments III and IV, but the number of branches ranges less than in *listoni*. On segments III and IV it is often relatively large but may be much smaller ; it may be simple but is usually 2- or occasionally 3-branched on III ; on IV it may be simple but is usually 2- or 3-, occasionally 4-branched ; on V it is small and simple, or 2-, rarely 3-branched ;

on VI it is very small or minute and simple or occasionally 2-branched, and on VII minute and simple or 2-branched.

PUPA. This differs from that of *A. funestus*, type form, and resembles that of *A. listoni*, as described by Senevet (1930), as follows :—

Lateral spines, A, on segments V to VII markedly elongated, that on VI being quite half and that on VII two-thirds the length of succeeding segments. Bristle C is also much longer on these segments than in typical *funestus*, the length being about equal to that of two segments.

The number of branches of bristle B shows considerable differences from the conditions described for *listoni*.

MALE AND FEMALE. It is not yet possible to define exactly the limits of the adult characters of this form.

From Mr. Leeson's material, however, it appears possible that the subspecies differs from the type form of *funestus* as follows :—

Third vein frequently with dark scales confined to small basal and apical spots. It may, however, be much more extensively dark, possibly in extreme cases, entirely dark.

Male palps with pale spots frequently (possibly always) present at base of club ; the spot is variable in extent and sometimes represented by one or two pale scales only. The constant absence of a pale interruption at the base of the costa has been noted above but this does not separate subspecies *leesoni* from Southern Rhodesian representatives of the type form.

Seasonal variation. Leeson's detailed study of variation in the wing markings of *funestus* probably applies mainly to this form and his conclusion that the specimens with the very pale third vein are most prevalent in the wet season indicates that the subspecies is subject to considerable seasonal variation. He also notes that more males than females showed the very pale third vein.

Described from 64 well-developed fourth stage larvae, including one larval pelt, also a pupal pelt and two pupae contained in 18 collections made in Southern Rhodesia, 1927-1928, by Mr. H. S. Leeson, who kindly supplied full data concerning them. In the following records the number of larvae from each type of breeding place are indicated by figures in brackets. Sinoia : stream beds (14) ; small stream with slowly moving water (6) ; water

standing in road (3 larvae, 2 pupae); pool (1); pond (1); cattle hoof-prints (8); Shamva: puddle in stream bed (1); edge of stream (1 larval and pupal pelt); roadside drain (1); near Salisbury: stream (1); Binduru: stream bed (31).

In two collections from Sinoia, from a stream bed and a pool, the larvae were associated with those of the type form of *funestus*. In nearly all cases the larvae were associated with those of other species of Anophelines, the species in order of frequency being *A. mauritianus*, *A. pretoriensis* and *A. rufipes*, *A. rhodesiensis*, *A. squamosus*, *A. costalis*.

Adults. An examination was made of 20 males, apparently of this form, collected by Mr. Leeson, from various indoor and outdoor situations in and near Shamva, Southern Rhodesia, 1927-1928.

The subspecies is named in honour of Mr. H. S. Leeson, who has added so greatly to our knowledge of the Anophelines of Southern Rhodesia.

LARVAE INTERMEDIATE BETWEEN *A. FUNESTUS*, type form, AND *A. FUNESTUS* subsp. *LEESONI*.

One specimen possessed the head characters of the type form together with the abdominal characters of subspecies *leesoni*. In another, the head possessed the transverse anterior band of the type form but the posterior frontal hairs were branched (7); its tergal plates were so faintly chitinised that it was impossible to decide their limits. The specimens were taken in a puddle, Fungwi Reserve, and associated with subspecies *leesoni* in a stream bed, Sinoia.

ANOPHELES FUNESTUS Giles. Type form.

Seasonal variation. The examination of a limited amount of material collected and bred in Sierra Leone, the type locality of this species, strongly indicates that in this region the colouration of the third vein is subject to seasonal variation analogous to that shown by Leeson (1930) to occur in South African forms.

With regard to the character of the female palps given by Christophers and Puri (1931), it should be pointed out that while, contrary to my figures (1927) the subapical band is typically very narrow, yet specimens do occur in which this is broader, being very little narrower than the intervening dark band.

Of the four larvae from Southern Rhodesia referable to the type form of *funestus*, none showed the anterior transverse band nearly as broad or well developed as in typical specimens from West and East Africa. The tergal plates were much paler than in typical examples of *funestus* and in one specimen the posterior frontal hair was bifurcated on one side. The specimens were collected at Sinoia (3) and near Salisbury (1).

The wing markings of the adults from Southern Rhodesia, as already noted, differ from the West and East African *funestus* in at least one character, the absence in all specimens of the basal costal interruption.

From the characters recently given by De Meillon (1931) it would appear that the larva of the form occurring commonly in the Transvaal, Zululand and Natal, agrees with the type form of *funestus*, at least with regard to the character of the tergal plates. The males, on the other hand, obviously differ very considerably from the type form, the third vein being mainly pale in all specimens. *A. funestus* from these regions would appear, therefore, to differ in certain details from both the type form and subspecies *leesoni*. A comparison between the larvae and adults and those of the two forms here dealt with will, doubtless, give extremely interesting results.

Conclusion. There is obviously a considerable amount of investigation to be done before the relationship of *A. funestus*, subspecies *leesoni* and other forms of *funestus* occurring in South Africa, including var. *fuscivenosus* Leeson, to the type form as seen in West and East Africa can be established.

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MISCELLANEA

OESOPHAGOSTOMUM CONICUM

In the Records of the Indian Museum, 1930, Vol. XXXII, p. 90, I named a new *Oesophagostomum* species from the pig, *Oesophagostomum conicum.* Dr. Benjamin Schwartz in a letter has kindly pointed out that this name is preoccupied. I therefore propose *Oesophagostomum conoides* to replace the above name.

P. A. MAPLESTONE

ONCHOCERCELLA nom. nov.

SYNONYM—*Katanga* Yorke and Maplestone, 1926, preoccupied.
Grammophora Gedoelst, 1916, preoccupied

In ' Nematode Parasites of Vertebrates ' we proposed the generic name *Katanga* to replace that of *Grammophora* given by Gedoelst in 1916, to the genus created by him for a filariid worm, *Grammophora katangensis,* found in *Petrodromus tetradactylus. Grammophora* was preoccupied, as it had previously been employed by Guénée, 1852, for a lepidoptera.

Dr. H. A. Baylis has recently been good enough to inform us that *Katanga* is also preoccupied as it was previously used by Schouteden in 1903 for a genus of *Reduviidae.* We therefore propose the name *Onchocercella* to replace *Katanga* Yorke and Maplestone, 1926, and *Grammophora* Gedoelst, 1916.

WARRINGTON YORKE.
16 *November,* 1931. PHILIP A. MAPLESTONE.

MALARIA IN PREGNANCY

On July 31, 1931, a lady, seven months pregnant, was admitted to hospital. She had returned from West Africa several months previously and gave a history of a number of slight attacks of malaria during this period.

The following notes on the subsequent history of the case are of interest :—

July 31. On admission the general condition of the patient was excellent ; she was not anaemic and was apparently in good health. Blood examination revealed a moderate infection with *P. falciparum*. During the evening she had a febrile disturbance, the temperature rising to 102° F.

August 1 to 9. During this period the patient was given daily (by the mouth) a mixture containing 15 grs. of quinine. The parasites rapidly disappeared from the blood ; and, as there was no further rise of temperature, the patient was discharged from hospital on August 9. Her doctor was requested to give her a mixture containing 15 grs. of quinine every Saturday and Sunday, to keep a careful record of her temperature, and to send a blood film for examination every Friday.

August 10 to 13. Patient well ; no rise of temperature.
August 14. Blood negative ; temperature normal.
August 15 and 16. Quinine, grs. 15, daily ; temperature normal.
August 17 to 20. Patient well ; temperature normal.
August 21. Patient felt unwell ; temperature 99° F. ; blood contained *P. falciparum*. Quinine, grs. 15.
August 22. Temperature rose to 99½° F. Quinine, grs. 15.
August 23. Temperature rose to 99½° F. ; severe headache. Quinine, grs. 15.
August 24. Patient better ; temperature normal. Quinine, grs. 15.
August 25 to 27. Patient remained well ; temperature normal.
August 28. Temperature normal ; blood negative.
August 29 to 30. Quinine, grs. 15, daily ; temperature remained normal.
August 31 to September 3. Patient well ; temperature normal.
September 4. Temperature normal ; blood negative.
September 5 to 6. Quinine, grs. 15, daily.
September 7 to 10. Patient well ; temperature normal.
September 11. Temperature normal ; blood contained *P. falciparum*.
September 12. Temperature rose to 100·2° F. Quinine, grs. 15.
September 13. Temperature normal. Quinine, grs. 15. Confinement.

The confinement was normal and a healthy full-term child was born. Pieces of the placenta were immediately fixed in Bouin's fluid and sent for examination. Sections showed a massive infection of the maternal sinuses with *P. falciparum* and much malaria pigment ; the blood in the foetal vessels of the chorionic villi was apparently uninfected.

The mother continued to take the week-end doses of quinine until November 8 ; she has remained free from attacks of malaria since the confinement, and various examinations of the blood have been negative. The child is apparently normal in all respects.

It is of interest to note that a course of quinine of moderate intensity—grs. 15 for nine consecutive days and thereafter grs. 15 every Saturday and Sunday until the confinement, which occurred five weeks later—failed to destroy a massive infection in the maternal

sinuses of the placenta, or to prevent occasional exacerbations of the disease with the reappearance of parasites in the peripheral blood.

It is, of course, impossible to state whether larger and more frequent doses of quinine would have sufficed to clear the placental blood and to prevent the relapses, or whether they would have had the result of producing a miscarriage; but from the point of view of the patient and her child the result of the procedure adopted seems to have been entirely satisfactory.

WARRINGTON YORKE.
FREDERICK MURGATROYD.

3 *December*, 1931.

The following have obtained the Diploma in Tropical Medicine of the University of Liverpool :—

Diploma in Tropical Medicine

Date of Diploma	
1904	Augustine, Henry Joshua
1904	Bennett, Arthur King
1904	Bruce, William James
1904	Byrne, John Scott
1904	Clayton, Thomas Morrison
1904	Dalziel, John McEwen
1904	Dee, Peter
1904	Greenidge, Oliver Campbell
1904	Hehir, Patrick
1904	Khan, Saiduzzafor
1904	Laurie, Robert
1904	Maclurkin, Alfred Robert
1904	McConnell, Robert Ernest
1904	Nicholson, James Edward
1904	Philipson, Nicholas
1904	Sharman, Eric Harding
1904	Thomson, Frank Wyville
1904	Walker, George Francis Clegg
1905	Anderson, Catherine Elmslie
1905	Brown, Alexander
1905	Caldwell, Thomas Cathcart
1905	Critien, Attilio
1905	Hooton, Alfred
1905	Hudson, Charles Tilson
1905	Illington, Edmund Moritz
1905	Macfarlane, Robert Maxwell
1905	Maddock, Edward Cecil Gordon
1905	Moore, James Jackson
1905	Nightingale, Samuel Shore
1905	Radcliffe, Percy Alexander Hurs
1905	Young, John Cameron
1906	Adie, Joseph Rosamond
1906	Arnold, Frank Arthur
1906	Bate, John Brabant
1906	Bennetts, Harold Graves
1906	Carter, Robert Markham
1906	Chisholm, James Alexander
1906	Clements, Robert William
1906	Dundas, James
1906	Faichnie, Norman
1906	Jeffreys, Herbert Castelman
1906	Mackenzie, Donald Francis
1906	Pailthorpe, Mary Elizabeth
1906	Palmer, Harold Thornbury
1906	Pearse, Albert
1906	Sampey, Alexander William
1906	Smithson, Arthur Ernest
1906	Taylor, Joseph van Someron
1906	Taylor, William Irwin
1906	Tynan, Edward Joseph
1906	Watson, Cecil Francis
1906	Willcocks, Roger Durant
1906	Williamson, George Alexander
1907	Allan, Alexander Smith
1907	Allwood, James Aldred
1907	Bond, Ashton
1907	Branch, Stanley
1907	Collinson, Walter Julius
1907	Davey, John Bernard
1907	Donaldson, Anson Scott
1907	Fell, Matthew Henry Gregson
1907	Gann, Thomas William Francis
1907	Graham, James Drummond
1907	Hiscock, Robert Carroll
1907	Keane, Joseph Gerald
1907	Kennan, Richard Henry
1907	Kenrick, William Hamilton
1907	Le Fanu, George Ernest Hugh
1907	Mackey, Charles
1907	Maddox, Ralph Henry
1907	McCarthy, John McDonald
1907	Raikes, Cuthbert Taunton
1907	Ryan, Joseph Charles
1907	Vallance, Hugh
1908	Caverhill, Austin Mack
1908	Crawford, Gilbert Stewart
1908	Dalal, Kaikhusroo Rustomji
1908	Dansey-Browning, George
1908	Davidson, James
1908	Dickson, John Rhodes
1908	Dowdall, Arthur Melville
1908	Glover, Henry Joseph
1908	Greaves, Francis Wood
1908	Goodbody, Cecil Maurice
1908	Harrison, James Herbert Hugh
1908	Joshi, Lemuel Lucas
1908	Le Fanu, Cecil Vivian
1908	Luethgen, Carl Wilhelm Ludwig
1908	Mama, Jamshed Byramji
1908	McCay, Frederick William
1908	McLellan, Samuel Wilson
1908	Pearce, Charles Ross
1908	Schoorel, Alexander Frederik
1908	Smith, John Macgregor
1908	Stewart, George Edward
1908	Tate, Gerald William
1908	Whyte, Robert
1909	Abercrombie, Rudolph George
1909	Allin, John Richard Percy
1909	Armstrong, Edward Randolph
1909	Barrow, Harold Percy Waller
1909	Beatty, Guy
1909	Carr-White, Percy
1909	Chevallier, Claude Lionel
1909	Clark, William Scott
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1909	Hanschell, Hother McCormick
1909	Hayward, William Davey
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1909	Innes, Francis Alexander
1909	Jackson, Arthur Frame
1909	Kaka, Sorabji Manekji
1909	McCabe-Dallas, Alfred Alexander Donald

Date of Diploma	
1909	Meldrum, William Percy
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1909	Shroff, Kawasjee Byramjee
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1909	Turkhud, Violet Ackroyd
1909	Webb, William Spinks
1909	Yen, Fu-Chun
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1910	Castellino, Louis
1910	Caulcrick, James Akilade
1910	Dowden, Richard
1910	Haigh, William Edwin
1910	Hamilton, Henry Fleming
1910	Hefferman, William St. Michael
1910	Hipwell, Abraham
1910	Homer, Jonathan
1910	Houston, William Mitchell
1910	James, William Robert Wallace
1910	Johnstone, David Patrick
1910	Korke, Vishnu Tatyaji
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1910	Macfie, John Wm. Scott
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1910	Murison, Cecil Charles
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1910	Nauss, Ralph Welty
1910	Oakley, Philip Douglas
1910	Pratt, Ishmael Charles
1910	Sabastian, Thiruchelvam
1910	Shaw, Hugh Thomas
1910	Sieger, Edward Louis
1910	Sousa, Pascal John de
1910	Souza, Antonio Bernardo de
1910	Waterhouse, John Howard
1910	White, Maurice Forbes
1911	Blacklock, Donald Breadalbane
1911	Brown, Frederick Forrest
1911	Chand, Diwan Jai
1911	Holmes, John Morgan
1911	Ievers, Charles Langley
1911	Iles, Charles Cochrane
1911	Ingram, Alexander
1911	Kirkwood, Thomas
1911	Knowles, Benjamin
1911	Liddle, George Marcus Berkeley
1911	Lomas, Emanuel Kenworthy
1911	Mackarell, William Wright
1911	MacKnight, Dundas Simpson
1911	Mascarenhas, Joseph Victor
1911	Murray, Ronald Roderick
1911	Oluwole, Akidiya Ladapo
1911	Rao, Koka Ahobala
1911	Sinton, John Alexander
1911	Tarapurvalla, Byramji Shavakshah
1911	Taylor, John Archibald
1911	Woods, William Medlicott
1912	Aeria, Joseph Reginald
1912	Anderson, Edmund Litchfield
1912	Borle, James
1912	Bowie, John Tait
1912	Brassey, Laurence Percival

Date of Diploma	
1912	Christie, David
1912	Dillon, Henry de Courcy
1912	Dunn, Lillie Eleanor
1912	Hardwicke, Charles
1912	Jagose, Jamshed Rustomji
1912	Kochhar, Mela Ram
1912	McGusty, Victor William Tighe
1912	Milne, Arthur James
1912	Mitra, Manmatha Nath
1912	Myles, Charles Duncan
1912	Pelly, Huntly Nevins
1912	Prasad, Bindeshwari
1912	Prentice, George
1912	Ross, Frank
1912	Russell, Alexander James Hutchison
1912	Ruthven, Morton Wood
1912	Sandilands, John
1912	Seddon, Harold
1912	Smalley, James
1912	Strickland, Percy Charles Hutchison
1912	Watson, William Russel
1913	Austin, Charles Miller
1913	Banker, Shiavux Sorabji
1913	Becker, Johann Gerhardus
1913	Carrasco, Milton
1913	Clark, James McKillican
1913	Forsyth, Charles
1913	Grahame, Malcolm Claude Russell
1913	Grieve, Kelburne King
1913	Hargreaves, Alfred Ridley
1913	Hepper, Evelyn Charles
1913	Hiranand, Pandit
1913	Jackson, Oswald Egbert
1913	Khaw, Ignatius Oo Kek
1913	MacKelvie, Maxwell
1913	MacKinnon, John MacPhail
1913	Macmillan, Robert James Alan
1913	Mouat-Biggs, Charles Edward Forbes
1913	Noronha, John Carmel
1913	O'Connor, Edward
1913	Olubomi-Beckley, Emanuel
1913	Pestonji, Ardeshir Behramshah
1913	Puttanna, Dodballapur Sivappa
1913	Reford, John Hope
1913	Smith, Edward Arthur
1913	Stewart, Samuel Dudley
1913	Walker, Frederick Dearden
1913	Wilbe, Ernest Edward
1913	Wilson, Hubert Francis
1913	Yin, Ulg Ba
1913	Young, William Alexander
1914	Arculli, Hassan el
1914	Chohan, Noormahomed Kasembha
1914	Connell, Harry Bertram
1914	Gerrard, Herbert Shaw
1914	Gimi, Hirji Dorabji
1914	Gwynne, Joseph Robert
1914	Hodkinson, Samuel Paterson
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1914	Kaushash, Ram Chander
1914	Kelsall, Charles
1914	Luanco y Cuenca, Maximino
1914	Misbah, Abdul-Ghani Naguib

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1914	Naidu, Bangalore Pasupulati Balakrishna
1914	Rowe, John Joseph Stephen
1914	Roy, Raghu Nath
1914	Shiveshwarkar, Ramchandra Vishnu
1914	Sur, Sachindra Nath
1914	Talati, Dadabhai Cursedji
1914	Wilkinson, Arthur Geden
1914	Wright, Ernest Jenner
1915	Lobo, John Francis
1915	Madhok, Gopal Dass
1915	Pearson, George Howorth
1915	Swami, Karumuri Virabhadra
1915	Wood, John
1916	Barseghian, Mesroob
1916	Chaliha, Lakshmi Prasad
1916	Lim, Albert Liat Juay
1916	Lim, Harold Liat Hin
1916	Metzger, George Nathaniel
1916	Söderström, Erik Daniel
1916	Wheeler, Louis
1917	Chapman, Herbert Owen
1917	Krishnamoorthy, Yedatore Venkoba
1917	Lipkin, Isaac Jacob
1918	Watts, Rattan Claud
1919	Bowle-Evans, Charles Harford
1919	Burnie, Robert McColl
1919	Celestin, Louis Abel
1919	Cummings, Eustace Henry Taylor
1919	Darling, Georgina Renington
1919	Drake, Joan Margaret Fraser
1919	Fraser, William James
1919	Gordon, Rupert Montgomerp
1919	Krige, Christian Frederick
1919	Maplestone, Philip Alan
1919	Oluwole, Isaac Ladipo
1919	Rustomjee, Khusshuyee Jamesidjee
1919	Sawers, William Campbell
1919	Thompson, Mary Georgina
1919	Turner, Gladys Maude
1919	Young, Charles James
1920	Adler, Saul
1920	Anderson, William Jenkins Webb
1920	Campbell, George
1920	Cobb, Charles Eric
1920	Cobb, Enid Margaret Mary
1920	Connolly, Evelyn Mary
1920	Fernandez, Daniel David
1920	Lim, Chong Eang
1920	McHutchison, George Browne
1920	van der Merwe, Frederick
1920	O'Farrell, Patrick Theodore Joseph
1920	Renner, Edowo Awunor
1920	Vaughan, James Churchwill
1920	Waller, Harold William Leslie
1921	Allen, George Phillip Parmer
1921	Corfield, Charles Russell
1921	Hamid, Abdul
1921	Longhurst, Bell Wilmott
1921	Macvae, George Anthony
1921	Madan, Hans Raj
1921	Mulligan, William Percival
1921	Nixon, Robert
1921	Richmond, Arthur Stanley
1921	Shri Kent, Shamsher Singh
1921	Skinner, James Macgregor
1921	Stewart, Robert Bell
1921	Thomson, Marion
1922	Bhatia, Jagat Ram
1922	Cohen, Morris Joshua
1922	Crawford, Andrew Clemmey
1922	Gilmore, Edward Raymond
1922	Gracias, Cajetan Manuel
1922	Jennings, Arthur Richard
1922	Lethem, William Ashley
1922	Paul, Sachchidananda Hoshen
1922	Pinder, John
1922	Rieley, Stanley Desmond
1922	Rutherford, Gladys
1922	Stewart, Quintin
1923	Abelman, B.
1923	Basu, Dhirendranath
1923	Cruickshank, John Cecil
1923	Doherty, Winifred Irene
1923	Edghill, Winifred M.
1923	Elsohn, John
1923	Fraser, N. D.
1923	Lee, R.
1923	Pierce, E. R.
1923	Raja, Rojaporum
1923	Reid, C. B. B.
1923	Richmond, A. E.
1923	Steven, J. B.
1923	White, Charles Francis
1924	Bilimoria, H. S.
1924	Carson, J. C.
1924	Chopra, B. L.
1924	Davis, B. L.
1924	Hardy, M. J.
1924	Jennings, C. B.
1924	Johnstone, F. J. C.
1924	Keirans, J. J.
1924	Lee, S. W. T.
1924	Macdonald, G.
1924	Maclean, G.
1924	Mathur, W. C.
1924	Mitchell, J. M.
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1924	Singh, H.
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1925	Adams, Alfred Robert Davies
1925	Ashton, Frank Richard
1925	Ashworth, Esther
1925	Bamford, Charles Walker
1925	Beinashowitz, Jack
1925	Black, John
1925	Clark, George
1925	Coghlan, Bernard A.
1925	Collier, Ivy
1925	Crawford, E. J.
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